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INVESTIGATION OF AQUEOUS SOLUTIONS OF SUCROSE, D-GLUCOSE AND D-FRUCTOSE WITH POSITRON LIFETIME SPECTROSCOPY

K. SÜVEGH[a], F. MOHOS[b] and A. VÉRTES[a]

[a] Laboratory of Nuclear Chemistry, Eötvös Loránd University, H-1518 Budapest P.O.Box 32. Hungary
[b] Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary

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Positron lifetime spectroscopy was applied to investigate aqueous solutions of sucrose, D-glucose and D-fructose. Lifetime spectra were recorded on water–sugar solutions of different compositions to study the structural changes of the water caused by the dissolved sugar molecules. The enzymatic inversion of sucrose in solutions of different sucrose-concentrations was investigated, as well.

Measuring the positron lifetime spectra we could monitore the size of the "free-volumes" of the water–sugar solutions. On the basis of our measurements we are able to state that positron lifetime spectroscopy can be a powerful method for the detection of changes of the hydrogen-bond structure of water caused by dissolved molecules. As our results suggested, these changes affect the size of the "free-volumes" of the solution. Using the free-volume theory of positron annihilation a simple model could be constructed to explain the observed structural modifications of the sugar solutions.

Keywords: sucrose solutions, aqueous solutions, positron lifetime spectroscopy

The water–sugar solutions of different composition are one of the most important components of several foods. They have been commonly used and investigated at least for a century: Tollens, 1876; Powell, 1914; Barbet, 1878; Smolenski and Kozlowski, 1931; Metthiesen, 1989 (TIMMERMANS, 1960). Density, viscosity and surface tension measurements were made on numerous solutions as a function of temperature and concentration of sugar. Moreover, diffractometry proved to be a very effective tool for the determination of sugar concentration. But, despite of the enormous number of measurements, one hardly knows how the dissolved sugar molecules affect the structure of water.

It is well known that H_2O molecules form hydrogen-bounded groups in water. On the other hand, IR-measurement showed that these groups had more or less definite structures. The other well-known fact is that the dissolved sugar affects the structure of water. A dissolved sucrose molecule, for example, can produce a H-bound cover of H_2O molecules around itself which obviously modifies the natural arrangement of water molecules. The study of this rearrangement can be useful in several cases but it needs a method particularly sensitive to the changes of the water structure.

Positron lifetime spectroscopy is a microscopical method for characterization of matter. When a positron annihilates with an electron the parameters of annihilation are affected exclusively by the immediate surroundings of this positron. The most commonly studied annihilation parameter is the period between the "birth" and the decay of the positron (i.e. the lifetime) which carries information about the electron density of the surroundings of the annihilation thus about the studied material itself. Moreover, positrons can take part in chemical reactions with the molecules of the studied material and these reactions are characteristic of the material, too.

A common positron source emits positrons with a fairly high kinetic energy (0.5–1 MeV). When such a positron enters a material it can cause significant changes till it looses its energy and becomes "thermalized". For thermalization of positron in fluids, a theory borrowed from radiation chemistry has proved to be suitable. It is the so-called "spur-model" (MOGENSEN, 1974).

This model assumes that positron colliding with electrons on its way produces electrons and different kinds of ions and radicals depending on the studied material. Any further phenomenon should take place between positrons and/or the radiation products. During its lifetime a positron can act as any other reactant agents can. E.g. it can form bound-state with molecules, it can participate in oxidation processes and it can avoid any reaction and freely annihilate, as well. (Sometimes these reactions are referred to as different positron states.) All these reactions can strongly affect the lifetime of positrons, consequently, they can be detected by the means of the lifetime spectra.

The most important reaction of a positron in fluids is the formation of positronium (Ps) atoms. The Ps-atom is a light atom which is a bound state between an electron and a positron. It has two basic states depending on the spins of the constituent particles. In the para-positronium (p-Ps), the spins of the electron and the positron are parallel, however, in the ortho-positronium (o-Ps) they are antiparallel. The lifetime-values (in vacuum) are characteristic: τ(p-Ps) = 120 picoseconds (ps) and τ(o-Ps) = 140 nanoseconds (ns). Because of its long lifetime only the o-Ps atom is usually taken into consideration during the evaluation of lifetime spectra.

The lifetime of o-Ps atoms are much less in fluids than in vacuum due to the reactions between the positronium atoms and the molecules of the fluid and due to the so-called "pick-off" annihilation (VÉRTES & KISS, 1987). The probability of the latter depends on the electron density. From the decreasing of the o-Ps lifetime, it is possible to make conclusions about the structural changes of the fluid. For example, density or surface tension parameters of a fluid are in direct correlation with the o-Ps lifetime (LÉVAY et al., 1973).

In most cases, the intensity of the positronium formation, i.e. the relative fraction of positrons forming positronium can provide additional informations.

Since the positrons decaying in a fluid originate from different positron-states, it is obvious that the measured lifetime spectrum [$S(t)$] is a superposition of the different decay curves:

$$S(t) = \sum_{i=1}^{N} I_i \lambda_i \exp(-\lambda_i t) \tag{1}$$

where

I_i: the relative fraction of positrons annihilating from the i-th state
λ_i: the annihilation rate of positrons in the i-th state (s^{-1})
t: the time variable (s)
N: the number of the states

In principle N is, for technical reasons, assumed to be not higher than four.

The measured spectrum is a convolution of the real spectrum [$S(t)$] and an instrumental time-resolution curve [$G(t)$], the latter in the simplest case is a Gaussian-function:

$$P(t) = G(t)\ \theta S(t)\,, \quad \text{i.e.} \tag{2a}$$

$$P(t) = \int_{-\infty}^{+\infty} G(\tau)\ S(t-\tau)\ \mathrm{d}\tau \tag{2b}$$

where

θ: the sign of convolution

A typical spectrum measured on water can be seen in Fig. 1.

1. Materials and methods

1.1. Materials

A positron lifetime spectroscopy study has been performed on aqueous sucrose solutions the concentrations of which varied between 0 and about 2 mol l^{-1}. In order to benefit the explanation of the results, aqueous solutions

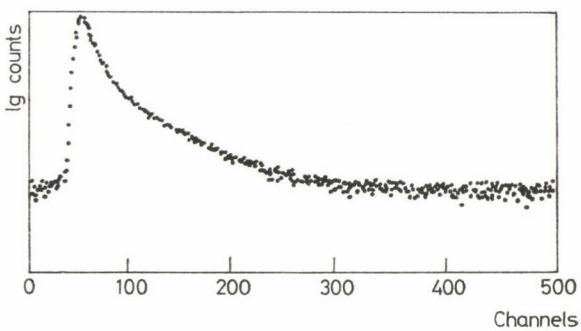

Fig. 1. Positron lifetime spectrum of water. Fast-slow coincidence system, ^{22}Na source diffused into glass foil. The spectrum was collected into 512 channels of a multichannel analyser. The time/channel value was 0.0425 nanoseconds

of D-glucose and D-fructose have been investigated in the concentration range from 0 to 2.8 mol l^{-1}, as well. The solutions were prepared from components of analytical grade and double-distilled water, respectively.

Some "quasi" time-dependent lifetime measurements were carried out, too, in order to follow the enzymatic inversion of sucrose in aqueous colutions of two different concentrations. The concentration of invertase was 0.5 g l^{-1} in both cases while the sucrose concentration were 1 mol l^{-1} and 2 mol l^{-1}, respectively.

1.2. Methods

The positron lifetime spectrometer was a fast-slow coincidence system (VÉRTES & KISS, 1987) with a time-resolution of about 310 ps (full width at half maximum of the resolution curve). The positron-source was prepared of ^{22}Na ($\sim$1 MBq) diffused into a thin ($\sim$2 mg cm^{-1}) glass-foil. According to the measurements only a small fraction of positrons ($< 5\%$) annihilated in the source itself, thus the source correction could be neglected during the evaluation of spectra.

To evaluate the lifetime spectra (Equation 2a and 2b), the POSITRONFIT EXTENDED computer program (KIRKEGAARD et al., 1988) was used which deconvoluted $P(t)$, and provided τ_i and I_i as final results. To collect a reliable spectrum during the time dependent measurements took about 150 min, therefore the changes in this long interval were integrated in the results. The time-points on the figures are calculated as the half-values between the starting and the terminal time-points of the measurement.

All the measurements were carried out at room temperature and normal pressure.

2. Results

Every spectrum could well be fitted with three lifetime terms. In the case of pure water, the lifetime parameters were very similar ($\tau_1 = 215$ ps; $\tau_2 = 450$ ps; $\tau_3 = 1820$ ps; $I_1 = 26\%$; $I_2 = 50\%$; $I_3 = 24\%$) to those given in the literature (ELDRUP et al., 1972). The two shorter lifetimes and their intensities did not change significantly in any case. Nevertheless, τ_3 and I_3 showed surprising changes during both the concentration-dependent and the time-dependent sets of measurement. The observed changes were slight but significant.

2.1. Concentration-dependence

The o-Ps lifetime and relative intensities measured on the aqueous solutions of three different types of sugars can be seen in Fig. 2. The shape of τ_3 curves are very similar for all the studied solutions. First the o-Ps lifetime in-

creases about 100 ps while the concentration of sugar increases from 0 mol l^{-1} to about 0.5 mol l^{-1} τ_3 reaches its maximum between the concentrations of about 0.5 mol l^{-1} and 1.0 mol l^{-1} then it begins to decrease. This decrease is the fastest in the case of sucrose. Some differences can be observed between sucrose and the smaller sugars according to the I_3-curves, too. I_3 has only a very slight, almost negligible increase in the range of low concentrations. On the other hand, if the concentration becomes higher than about 1 mol l^{-1} I_3-values decrease significantly for sucrose but remain stable for the other sugars.

A maximum of positronium lifetime versus concentration is a surprising phenomenon. As it is well-known, any macroscopic parameter of sugar solution is a monotonous function of concentration. Moreover, density measurements on sucrose solutions (see Table 1) were in parallel performed, and the

Table 1

Density of sucrose solutions at 20 °C

	Sucrose concentration (mol dm^{-3})					
	0.2	0.35	0.5	0.8	1.1	1.5
Density (g cm^{-3})	1.02162	1.04305	1.06231	1.09889	1.13700	1.17127

observed monotonous change of density can not cause any maximum behaviour of the o-Ps lifetime.

Applying the free-volume model of the positronium formation (BRANDT et al., 1960), a possible explanation for the above described maxima of τ_3- and I_3-curves can be constructed. According to this model, every material has more or less free-volumes between its molecules. The size of these free-volumes has a very large influence on the o-Ps lifetime, while the number of such "empty spaces" affects the Ps-formation intensity.

In pure water there are hydrogen-bound H_2O molecules determining a particular structure. This structure contains empty spaces, too, between the H_2O molecules where the positronium atoms can form and "live". The lifetime and the intensity of o-Ps depend upon the size and the amount of these "free-volumes". Adding sugar to the aqueous solutions, the original arrangement of H_2O-molecules can break by the sugar molecules. At first, as the τ_3-curves show, the size of the free-volumes in this new arrangement can be larger than in the water itself.

A further addition of sugar should lead to a re-arrangement of the structure which contains less free space. That is before the lifetime of the o-Ps reaches its maxima the sugar-addition can disturb the original structure of the pure water and can disrupt the hydrogen-bridges of it. Therefore, the free-volume in the water structure and the lifetime of the o-Ps can increase. After

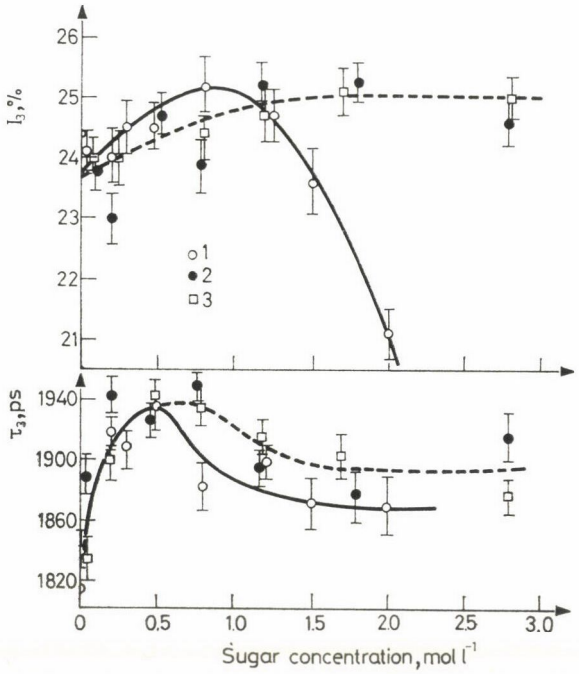

Fig. 2. τ_3 and I_3 values versus the sugar concentration. The depicted values were evaluated from the measured spectra by POSITRONFIT EXTENDED computer program. Every spectrum contained approximately one million counts to provide proper statistics. D-glucose (-----□-----), D-fructose (——●——) and sucrose (——○——)

the maxima, the further addition of sugar can increase the amount of the H and OH groups in the solution, consequently, hydrogen-bridges can form again, thus the free-volume and the lifetime of the o-Ps decrease.

The assumption that the changes of the annihilation parameters of o-Ps are caused by the ordering of the H_2O molecules, can be confirmed by the τ_3-curves measured at different sugar solutions. Because sucrose is twice as big as glucose or fructose are it can make twice as much hydrogen bound with water molecules as glucose or fructose do. Therefore a lower amount of sucrose can cause a similar arrangement than a certain amount of glucose can.

2.2. Time-dependence

The inversion of the two solutions of sucrose gave two different types of results. τ_3- and I_3-values of the saturated solution showed extremes, but the same parameters of the diluted solution monotonously changed.

These curves are in accordance with the above assumption about the role of the free-volume: The saturated solution is highly ordered. When the enzyme starts to split the sucrose molecules into glucose and fructose the

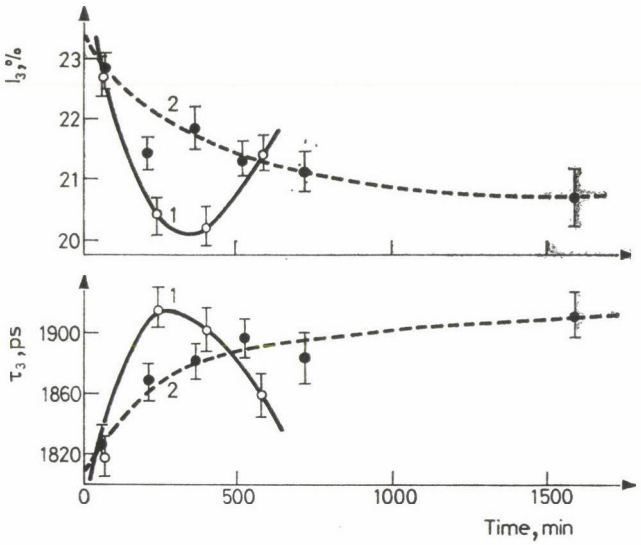

Fig. 3. Time-dependence of τ_3 and I_3 during the inversion procedure. Every referred spectrum was measured in 150 min time-periods. The time values on this figure are the means of the measuring periods. Saturated (—o—) and "diluted" (—●—) sucrose solutions

arrangement of H_2O molecules changes. The break of this structure can form larger free-volumes, in this case. On the other hand, the solution has not enough free H_2O molecules, thus the formation of a new structure starts after a certain period, only. This new structure decreases the free-volume thus the lifetime of o-Ps atoms.

The diluted solution is far from the highly ordered structure and the supernumerary H_2O molecules allow the new glucose and fructose molecules to form hydrate covers without the formation of an ordered structure. During the inversion the number of the sugar molecules, thus the number of the separated hydrate-covers, increases. The formation of new hydrate-covers can continuously modify the original structure of the solution.

At this point, the assumption that any reaction of o-Ps or positron could play a provoking role in the development of the phenomena discussed here, can be excluded. In the time-dependent measurements there were no changes in the concentration of sugar. Therefore, assuming a reaction of o-Ps atoms as a cause of the observed changes, no change should have been observed in this series of measurements.

In both cases, the changes of I_3-values are in good agreement with those of τ_3-values and the free-volume theory. If the size of free-volumes increases their amount decreases and this fact provides a decreasing probability of the formation of o-Ps atoms.

All these can be seen in Fig. 3.

3. Conslusions

On the bases of the presented positron lifetime measurements, a simple model could be created for a possible explanation of the influence of sugar molecules on the structure of water.

The authors assume that sugar molecules break the original hydrogen-bond structure and increase the free-volume in the aqueous solutions. After reaching a certain concentration, a new arrangement of the hydrogen-bound molecules begins to form which descreases the free-volume in the solution again. This model fits the obtained results satisfactorily but needs a further confirmation by the means of other methods.

The measurements on the time-dependent inversion showed that no inhibition effect or chemical reaction of o-Ps could cause the changes observed.

The structure of water and the aqueous solutions is one of the most important topics both in chemistry and in the food-science, consequently, our knowledge on it has unceasingly to be enlarged. The present work, with its modest means intends to contribute to this relevant task.

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TOXICOLOGICAL EVALUATION OF POLLEN MULTIFLOWER

M. Abreu, A. Castillo, T. Gonzalez,
I. Farras and R. Gomez

Institute of Nutrition and Food Hygiene, Infanta 1158, Havana. Cuba

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Some toxic or antinutritional factors were determined in pollen multiflower. Its safety was tested in rats by a subchronic toxicological assay. Alkaloids, saponins, cyanide and trypsin inhibitors and hemagglutinating activity were negative. Polyphenols content was very low. The data obtained in the study did not reveal severe damage to the rats due to the pollen feeding.

Keywords: pollen, rats, toxicological evaluation

The pollen collected by the honey bees is a vegetable product that is used as food by the population of some rural regions (Cabrera, 1979). Its proteins show a well balanced amino acid pattern with an Amino Acid Score of 82% for tryptophan and a Biological Value of 84%. Only the digestibility of the proteins is relatively low. In addition, pollen shows a good mineral and fatty acid composition with 68% of unsaturated fatty acids (Abreu, 1989).

In tropical regions the honey bee-collected pollen that could be produced is potentially enormous, and has to be taken into consideration if a high level of production is undertaken, however the introduction of pollen as a human food source requires further research on its safety. With this objective, the presence of some toxic or antinutritional factors were determined in the pollen multiflower and a subchronic toxic assay was done in rats fed on it.

1. Materials and methods

The honey bee-collected pollen was supplied by the Agriculture Department of the Ministry of Agriculture of Cuba from commercial production.

The presence of alkaloids (Macked, 1972), saponins (Rondina & Caussio, 1969) and cyanide (Burriel, 1968) were qualitatively assayed. Trypsin inhibitors (Kakade, et al., 1969), hemagglutinating activity (Jaffé & Brucher, 1972) and polyphenols content (Dev Choudhury & Goswami, 1983) were also determined.

The pollen multiflower was subjected to a 3-month subchronic toxicological assay which was designed according to the recommendation of the Protein Advisory Group (PAG) for the evaluation of new protein sources (PAG/UNU, 1984).

Four groups of 10 rats (5 male and 5 female) weighing 65–70 g were formed and housed in collective cages at room temperature and normal humidity. Groups 1 and 2 received respectively casein and a commercial diet, group 3 was fed on a pollen-based diet, in which 50% of the protein was supplied by casein and group 4 received pollen as the only source of protein. The diet formulation and analysis are shown in Table 1. Food and water were provided ad libitum. The individual body weight was recorded once a week.

Table 1
Composition of the experimental diets in the subchronic toxicological assay

Diet	Composition of diets (%)							Assay	
	casein	pollen	corn oil	mineral mixture[a]	vitamin mixture[a]	cellulose	autoclaved corn starch	Proteins (N×6.25) (%)	Energy (KJ g^{-1})
Casein	24	—	8	5	1	5	57	19.2	19.2
Commercial feed	—	—	—	—	—	—	—	15.0	14.6
Pollen 50%	11	50	5	5	1	—	28	18.3	19.2
Pollen 100%	—	94	—	5	1	—	—	17.9	19.2

Proximate composition of the pollen (%): moisture 17.7; ether extract 17.5; protein (N×6.25) 19.0; ash 2.9 and crude fibre 7.4
[a] According to AOAC, 1975

Three days before the end of the assays the rats were placed in metabolic cages in order to collect the urine of 24 h in which volume, pH, glucose, proteins, leucocytes and erythrocytes were measured. Diets were removed 12 h before the rats were to be killed by exsanguination from the femoral vein. Serum analysis involved hemoglobin, hematocrit, leucocytes, differential white-cell count, creatinine, total proteins, albumins and glutamic, pyruvic and glutamic oxaloacetic transaminase determinations (KAMOUN, 1985).

Liver, intestine, kidneys and spleen were immediately removed and weighed, and subjected to histopathological examination. Intestine was emptied from the pylorus to the ileocecal valvule and then weighed.

Lactic and succinate dehydrogenases and alkaline phosphatase activity were measured by histochemical examination of the liver and intestine (ELISEIEV et al., 1985).

Statistical analysis involved a double classification variance analysis (MATTHEWS & FAUWELL, 1988). When means were found to be significantly different the Duncan multiple range test was used to determine which means were significantly different from each other.

2. Results

Alkaloids, saponins and cyanide tests were negative, while no trypsin inhibitor and hemagglutinating activity were observed in the pollen. Polyphenols content (175 mg per 100 g of samples) was similar in range to the data normally reported for vegetable products (ABREU, 1987).

The body weight curves of the male rats fed on the casein and 50% pollen diets were very close, and higher than that of the commercial feed and 100% pollen groups (Fig. 1). The differences among the female groups were smaller (Fig. 2), however the 100% pollen group showed higher values than those on commercial feed.

The results of the hematological and serum biochemical assays are shown in Table 2. The data show some differences among the experimental groups, however these do not reveal a constant trend. The differences could be ascribed to the logical variations of an animal feeding experiment. In general, almost all results of the hematological and serum biochemical assays agreed with the normal range values reported for rats (WOLFOD et al., 1986). The most significant differences arose from the glutamic pyruvic transaminase assay. The values for the male and female groups of the commercial feed and 100% pollen were more than twice higher than those of the casein fed groups.

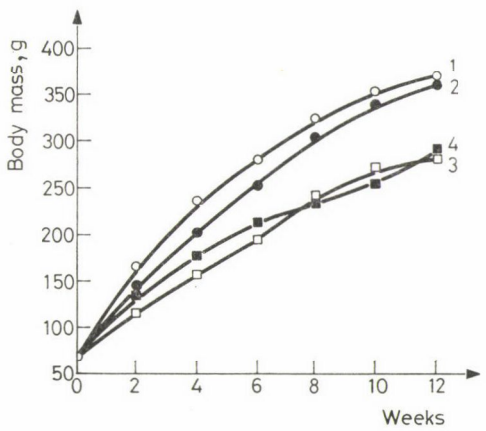

Fig. 1. Body weight of the male rats in the subchronic toxicological test. 1: C, casein; 2: P50%, pollen 50%; 3: P100%, pollen 100%; 4: CF, commercial feed

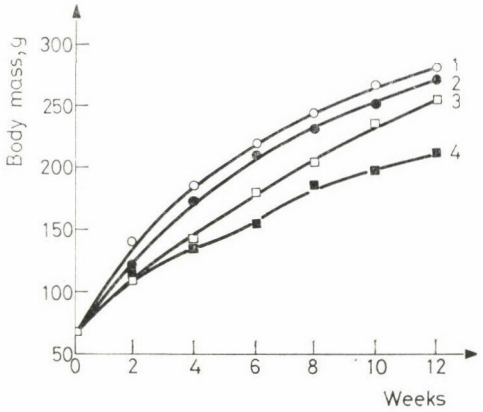

Fig. 2. Body weight of the female rats in the subchronic toxicological test. 1: C, casein; 2: P50%, pollen 50%; 3: P100%, pollen 100%; 4: CF, commercial feed

Total and differential white-cell count revealed no differences among the experimental groups. All values were in the normal range (WOLFOD et al., 1986), indicating that the immune system of the rats probably was not affected. No differences in the urine assays among the experimental groups were found either.

Table 3 shows the relative organ weight of the experimental groups. The liver and intestine weights for both sexes were always ranked in the following ascending order: casein, commercial feed, 50% pollen and 100% pollen, a trend that was not observed for kidneys and spleen.

The results of the histological examinations showed some reversible damage of the liver and kidneys of the experimental groups, including the controls of casein and commercial feed, which is in agreement with the experience of our Histopathology Department. However, slight inflammation of the liver was more frequently observed in the commercial feed and pollen groups than in the casein groups. Casein, commercial feed and pollen groups showed 20, 50 and 60% of liver inflammation, respectively.

Lactic and succinate dehydrogenases and alkaline phosphatase activity determined by histochemical examination revealed no differences among the experimental groups.

3. Discussions

The body weight curves (Figs. 1 and 2) were in agreement with the protein quality of the casein and pollen. Previously a Biological Value of 84% and an Amino Acid Score of 82% for tryptophan were found for the pollen, although its biological utilization could partially be limited by the low protein digestibility (79%) (ABREU, 1989). As was expected higher weight curves for the diet

Table 2

Hematological and serum biochemical results of the rats fed on the experimental diets in the subchronic toxicological assays

Group	S	Hemoglobin (g l^{-1})		Hematocrit (l)		Creatinine (μmol l^{-1})		Total proteins		Albumins (g l^{-1})		Globulins		GOT (IU l^{-1})		GPT (IU l^{-1})	
		$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$				
Casein	M	151	7 a	0.50	0.02 a	35	6 ab	77	7 a	52	8 a	27	5 a	6.4	2.2 ab	38	11 a
	F	143	9 ab	0.45	0.03 ab	29	4 b	77	4 a	52	4 a	28	5 a	4.0	0.1 b	40	2 a
CF	M	137	7 b	0.45	0.01 ab	60	16 c	60	4 b	40	3 bc	20	2 b	17.0	2.0 c	43	6 a
	F	141	10 ab	0.43	0.02 b	49	15 d	64	4 b	37	3 c	27	1 a	15.2	2.1 c	47	3 a
P 50%	M	147	4 ab	0.47	0.01 ab	31	5 b	75	7 ab	49	8 ab	26	1 a	6.0	2.0 ab	36	4 a
	F	137	8 b	0.43	0.02 b	40	11 ad	78	6 a	50	6 ab	28	1 a	9.8	4.0 a	37	2 a
P 100%	M	151	11 a	0.50	0.05 a	37	8 ab	76	4 a	51	3 a	26	1 a	15.2	4.0 c	37	4 a
	F	148	6 ab	0.45	0.03 ab	41	7 ad	85	4 c	58	2 a	27	3 a	15.0	2.7 c	42	7 a

$\bar{x}$: mean value; $\pm s$: standard deviation; CF: commercial feed; P: pollen; S: Sex (M: Male; F: Female); GPT: glutamic pyruvic transaminase; GOT: glutamic oxaloacetic transaminase. Means with different superscripts were significantly different ($P < 0.05$).

Table 3

Relative organ weight of the groups of rats fed on the experimental diets in the subchronic toxicological assays

Sample	Sex	g per 100 g of body weight							
		Liver		Intestine		Kidneys		Spleen	
		$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Casein	M	2.40	0.16 a	1.83	0.17 a	0.60	0.03 a	0.16	0.01 a
	F	2.40	0.27 a	2.20	0.15 b	0.57	0.02 a	0.18	0.01 ab
Commercial feed	M	2.41	0.12 a	2.13	0.24 b	0.54	0.04 b	0.15	0.01 a
	F	2.53	0.09 a	2.44	0.23 b	0.54	0.04 b	0.17	0.02 ab
Pollen 50%	M	2.68	0.12 ab	2.31	0.11 b	0.63	0.02 a	0.16	0.01 a
	F	2.64	0.15 ab	2.76	0.11 c	0.61	0.03 a	0.20	0.02 b
Pollen 100%	M	2.96	0.23 b	2.96	0.17 cd	0.62	0.02 a	0.14	0.01 c
	F	2.90	0.22 b	3.18	0.28 d	0.58	0.02 a	0.20	0.02 b

$\bar{x}$: mean value
±s: standard deviation
Means with different superscripts were significantly different ($P < 0.05$)

at 50% than those for the diet at 100% were obtained when casein was added to the tryptophan deficient pollen.

Body weight appears as a quite sensitive biological response of animal health, and is the resultant of many separate, but interconnected factors. The weight gain of the experimental groups, therefore, could indicate that the health of the rats was not grossly affected by the tested diets. In addition, the total and differential white-cell count suggested no alteration in the health of the rats.

However, the essential amino acid deficiencies of the pollen, even when they are not too high, could alter the appropriate growth and some metabolic and physiological functions of the rats, particularly because the rats were fed on the experimental diets for a long-term period (10% of their life span). Amino acids in excess over the most limiting ones are deaminated prior to their utilization as energy source, increasing transaminase activity and liver size (MUNRO, 1970). This would explain, at least partially, the high glutamic pyruvic transaminase level (Table 2) and the high liver weight (Table 3) found in these groups, however it is impossible to disregard the influence of other factors. These could arise from the detoxificating action of the liver in relation to some substances present in the ingested samples. Likewise, liver weight could also be affected by the body weight of the rats. Weight changes in some organs are not always in the same proportion as would be expected from the body weight.

In the light of what has been mentioned above it is reasonable to suggest that pollen had little, if any, adverse effect on the liver of the rats fed on it.

The relative weight of the intestine is affected by the level and type of dietetic fiber of the pollen. Different types of dietetic fiber gives an increase in

intestinal weight (JACOBS & SCHNEEMAN, 1981; JACOBS, 1985), results that are compatible with the increased DNA content also observed in the intestine of rats fed on different types of dietetic fiber (JACOBS, 1983).

4. Conclusions

The data obtained in the study did not reveal severe damage to the rats due to pollen feeding. This result suggests that the risks to humans of the ingestion of pollen under normal circumstances would be negligible, however its innocuousness has to be tested in humans.

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STORAGE TIME AS A FACTOR IN DETERMINING PHYSICO-CHEMICAL PARAMETERS OF NEW CHEESE PRODUCTS

LJ. M. LALIĆ and K. BERKOVIĆ

Faculty of Food and Biotechnology University of Zagreb
Pierottieva 6, Zagreb. Yugoslavia

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Changes of physico-chemical parameters have been determined for the fresh prepared samples of new products based on fresh cottage cheese, semolina and eggs with adding of sodium chloride, cooked chopped spinach, hot red pepper, sucrose, vanilla sugar and cinnamon, and for the samples stored at —14 °C. At definite periods of time the changes in electroconductivity values, pH values, degree of acidity and texture of the prepared and stored samples during frying, were determined as well as products were checked for homogeneity.

The amount of nitrate, nitrite and iron was established in cooked spinach and in the samples with the addition of spinach and cottage cheese amounting to the weight share of 25%.

The samples of the new products, were shaped as 40 g biscuits, packed in small low density PE folio sacks, 50 μm thick, and stored at 259 K. They were analysed after 20, 40, 60 and 70 days, having previously reached the temperature of 20 °C.

Keywords: new cheese products, changes in physico-chemical parameters, pH, degree of acidity, electroconductivity

Man's nourishment requires new products of higher quality and satisfying nutritive value, which also means a high biological value. Shelf-life of such products should be as long as possible and the time necessary for a semi-finished product to be prepared and transformed into a meal for consumption, the shortest.

Cottage cheese is one of the significant provisions in man's nourishment, therefore many studies have been conducted on processing and testing of its quality (DYACHENKO et al., 1984). Most authors emphasize the fact, that the obtained milk products — including milk compared with other products are rich in amino acids whose amount in food is especially important and necessary for man's life (SIPKA et al., 1975).

Beside being consumed as a final product, cheese is also used as an addition in meal preparation as well as in the preparation of new products.

Changes of physico-chemical parameters have been determined for the fresh prepared samples of new products based on fresh cottage cheese and for the samples stored at —14 °C. At definite periods of storage time the changes in electroconductivity values, pH values and degree of acidity were determined.

Akadémiai Kiadó, Budapest
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1. Materials and methods

The new products are prepared from fresh cottage cheese, semolina and eggs. To the base composition of new products the following ingredients may be added to make a new product (2, 3, 4, 5, 6 and 7 class samples):

— 2% sodium chloride	7 class samples
— 25% cooked chopped spinach	5 class samples
— 1.5% hot red paprika	6 class samples
— 12% sucrose	2 class samples
— 3% vanilla sugar	3 class samples
— 1.5% cinnamon	4 class samples

Freshly prepared product of homogenized and mixed components were shaped as 40 g biscuits, packed in small low density PE folio sacks, 50 μm thick and stored for 2 h in the refrigerator at 4 °C. After 2 h 1.5 cm thick cakes of about 40 g are formed, then stored at the temperature of —14 °C.

The samples were packed in welded bags and stored in the freezer at —14 °C.

Quality control by checking the organoleptic properties and the determination of physico-chemical parameters were carried out on fresh samples and after storage of 20, 40, 60 and 70 days. All samples were previously kept at the temperature of 20 °C, without any further treatment.

To establish acidity, pH and conductivity, the sample mass is used as weighed to $\pm$ 0.0009 g accuracy. A sample cut into small pieces is filled with distilled water and is sealed. The total volume is not larger than 100 cm^3. The acidity is determined by titration with decimolar solution of sodium hydroxide in the presence of phenolftalein to the stable rosy colour (SLUŽBENI LIST SFRJ, 1983). The pH value is determined with the digital pH-meter type 647 product of "Knick" (Yugoslavia).

Electroconductivity is determined by the conductometer "Iskra", Kranj by immersing the Ni-electrode into the sample and by direct marking of conductivity values in μS cm^{-1}.

2. Results and discussion

The examined samples are polycomponent systems whose physico-chemical properties are affected by many factors on the way from sample preparation to long-term storage. In the experiment the method of freezing was applied in order to keep the product fresh as long as possible, since its shelf-life is limited under normal storage conditions. The method of conservation

was proven appropriate, because the physico-chemical properties of the system were not disturbed during storage (KOSTYRA et al., 1981).

In these works five series of 30 samples of the new products were analysed. During a 70-day period five samples were taken from each series and changes of parameters recorded.

Figure 1 shows electroconductivity values for the cheese samples analysed, and newly-prepared products during 0–70 days storage period at —14 °C.

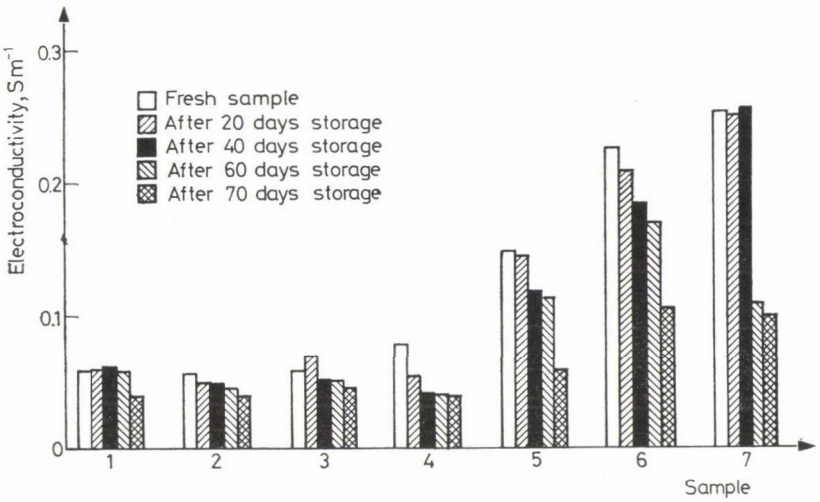

Fig. 1. Electroconductivity (μS m^{-1}) of new products (sample 2, 3, 4, 5, 6 and 7) and base composition of examined products (sample 1) on the first day and during 70 days storage

Judged by mean values for electroconductivity and coefficients of variation it can be concluded that the samples retained their quality during 70-day storage. Newly prepared samples with addition of sodium chloride, spinach or paprika, showed somewhat higher electroconductivity values than the basic cheese sample (Sample 1) as well as samples with added sucrose, vanilla sugar or cinnamon (Table 1).

Electroconductivity values decrease in all samples during storage. This decrease is somewhat less obvious in samples with added vanilla sugar and cinnamon which could be caused by subjective error in determination.

The tested samples have cottage cheese as their basis. Since it is known that milk and cheese conductivity, depends on the chloride ions present (ĐORĐEVIĆ, 1982), it is obvious that the samples with 2% sodium chloride content will have higher electroconductivity in relation to other samples. The decrease of electroconductivity in all samples at low temperature is caused by the change in the physico-chemical parameters of the products due to the decreased mobility of ions at low temperatures, SNOEREN and co-workers (1983),

Table 1

Coefficient of variation for electroconductivity values

No.	Sample stored at —14 °C	Storage time per days				
		0	20	40	60	70
1	Base compound cheese, eggs, semolina	1.40	1.70	0.98	2.69	2.59
2	Compound with 12% sucrose	1.55	1.47	5.28	2.22	2.07
3	Compound with 3% vanilla sugar	3.39	5.73	2.44	1.41	4.30
4	Compound with 1% cinnamon	1.77	0.59	1.77	1.77	1.63
5	Compound with 25% spinach	2.16	2.61	8.25	6.91	4.42
6	Compound with 1.5% ground hot red paprika	6.11	3.33	1.34	0.96	5.59
7	Compound with 2% sodium chloride	4.57	5.42	6.28	1.71	4.80

have proved working with concentrated milk that, depending on the percentage dry substance and on the volume of casein in tested samples, the viscosity value increases and the mobility decreases. Larger and more compact conglomerates are formed in the samples, increasing the mobility (explained by SNOEREN et al., 1984) by the formation of fibrillar casein micelles which influence the change in salt balance, thus, increasing also acidity. During storage acidity decreases in all samples, but increases in cheese and first-batch samples, due to fermentation processes.

Coefficients of variation determined on the basis of the known data for acidity also indicate satisfactory shelf-life of new products (Table 2).

Table 2

Coefficient of variation for acidity value

No.	Sample stored at —14 °C	Storage time per days				
		0	20	40	60	70
1	Base compound cheese, eggs, semolina	0.35	0.11	0.20	0.38	1.10
2	Compound with 25% sucrose	0.03	0.40	0.18	0.09	0.30
3	Compound with 3% vanilla sugar	0.07	0.07	0.14	0.02	0.18
4	Compound with 1% cinnamon	0.30	0.03	0.08	0.14	0.29
5	Compound with 25% spinach	0.03	0.19	0.24	0.97	1.38
6	Compound with 1.5% of ground hot red paprika	1.04	0.11	2.70	0.96	0.88
7	Compound with 2% sodium chloride	0.63	3.17	0.66	1.30	1.75

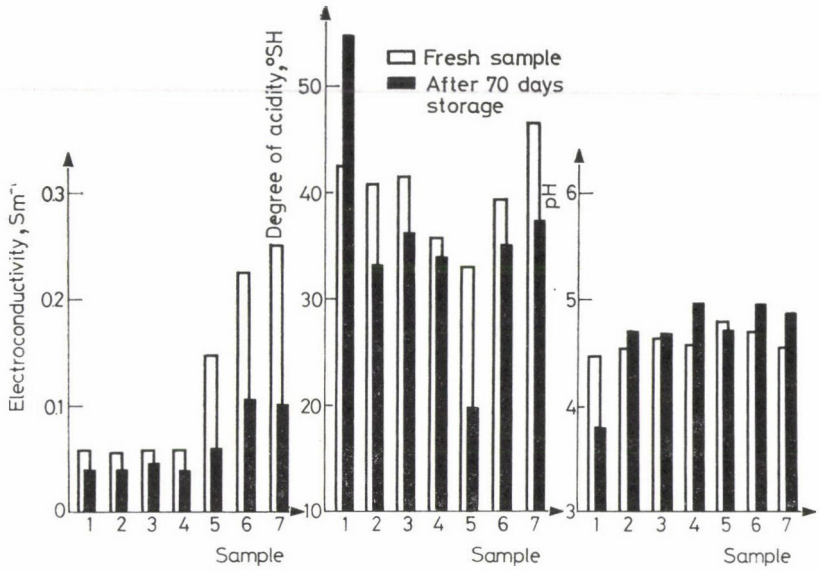

Fig. 2. Electroconductivity, degree of acidity and pH values of the fresh sample and samples during 70 days storage

The mean value of electroconductivity, SH and total acidity pH in Fig. 2, for all investigated samples and coefficients of variation of this value also indicate satisfactory shelf-life of these products.

Low temperatures during storage of the new products decrease the kinetics of the development reactions within complex systems proved by results of the applied measuring methods.

The determined values of electroconductivity, acidity and pH, correlated well and can be used for the characterization of investigated systems and control of their quality and usability after their storage.

Testing of organoleptic properties, colour determination and texture of the prepared and stored samples during storage and frying, as well as checking of products for homogeneity, have been carried out after storage time.

The amount of nitrate, nitrite and iron was estimated in cooked spinach and in the samples with the addition of spinach to cottage cheese amounting to 25% of the weight.

3. Conclusions

On the basis of testing the physico-chemical parameters of the fresh and new complex foods, polycomponent systems which are stored at low temperature (−14 °C) it can be concluded, that the applied methods can safely be used for the quality control of such products. There were no data

available in the literature to be correlated with data in this work, but obtained data can be used to characterize such complex food systems.

The decrease of electroconductivity in the samples during their storage was caused by the change of salt balance in the samples, which also indicates the decreased product stability.

Low temperatures during storage cause the kinetics of particular reactions in the samples at the beginning of storage to be slower, but after 40 days the changes in determined parameters increased.

The control of the amounts of nitrite and nitrate in the raw materials used in preparation of the new products has proved, that the products are appropriate and ready for consumption and contain larger amount of iron and more satisfying nutritive value.

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EFFECT OF HOME PREPARATIVE PROCEDURES AND TECHNOLOGICAL PROCESSES ON LINDANE RESIDUES IN TOMATO

B. A. A. Bessar, K. Korány and A. S. Szabó

University of Horticulture and Food Industry, Department of Food Chemistry and Nutrition, H-1118 Budapest, Somlói út 14–16. Hungary

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A method using gas liquid chromatography with electron capture detector (ECD) was used to follow the effect of washing and some preservation methods on lindane residues in tomato and in some tomato products immediately post treatment and 15 days later. Water washing and washing with detergent reduced lindane residues by 31.7% and 42.6% immediately after treatment and 15 days later by 19.9% and 55.9% of the initial amount present in unwashed samples. The perscribed waiting period of 15 days post treatment reduced the level of lindane residues to 23.9% of the initial amount found directly after treatment. All technological processes lowered lindane residues level from 19.9% to 50% immediately after treatment and from 17.5% to 100% after 15 days post treatment.

Keywords: lindane residues, tomato, GLC technique

In recent years, many agrochemicals were used in the field of plant production and animal production. Pesticides, as agrochemicals for plant protection are used widely throughout the world for pest control and decreasing the losses in the crops due to harmful pests. Man undoubtedly still would consume contaminated foods which contained various levels of different pesticide residues. Therefore, it is of great importance to minimize such levels of these pesticide residues remaining on and in plant foods to be within or below the acceptable daily intake and lower than the safety tolerance level for protection of human health by using home preparations like washing the fruit before eating it or by the effect of technological processes for plant foods such as sterilization, pasteurization, concentration by heating and tomato puree production.

1. Materials and methods

In this study lindane was used as chlorinated hydrocarbon insecticide taking an active part in pest control, and tomatoes which have a waxy skin and are eaten freshly or processed in to many preserved products. The mean recovery ratio was 92.98% and this value was used to correct all obtained values of lindane residues. Gas-chromatographic analysis of lindane residues was used.

1.1. Reagents

Lindane (γ-hexachlorocyclohexane), technical grade material is specified to be 99.9% pure of light white colour, acetone pure, benzene pure, dichloromethane (Merck No. 6050), n-hexane (Merck No. 9688). Elution mixture (10:2:2) dichloromethane–benzene–acetone, charcoal pure, (AR), (No. 2186), Cellite 545 (C. Roth Karlsruhe), silica gel 0.05–0.2 mm (Merck No. 7734), sodium chloride (AR) and anhydrous sodium sulphate.

1.2. Equipment

A Hewlett–Packard model 5730/A gas-liquid chromatograph equipped with a liner Ni^{63} electron capture detector (18713/A) and 2 cm stainless steel transfer line from the column to the detector was used for the lindane assays. A 1.5 mm (i.d.) 1.8 m glass column packed with 1.95% OV–17 on 100–120 mesh Chromosorb was used under the following conditions: Carrier gas 5% methane in argon at 60 cm^3 min^{-1} flow, injection port 250 °C; oven temperature 270 °C, detector temperature 300 °C, starting temperature 200 °C, speed of heating 2 °C min^{-1} and initial holding time 2 min. Injection of 1 μl using 10-μl Hamilton syringe equipped with change adapter.

1.3. Treatment

Ten kilograms of green tomatoes (from Budapest markets) were treated with 100 cm^3 of the field concentration of 0.6 g dm^{-3} lindane using Sigma Ultra Sprayer, where all the treated fruits coated with lindane film from all sides by trimming the fruits during the treatment. The treated fruits were left to dry in plastic trays and loosely covered with polyethylene bags. Treated fruits were divided to two equal parts, the first one used directly after lindane treatment and the second one kept for 15 days at 10 °C to simulate shipping and marketing conditions. Fruits of both parts were washed with tap water only, and with ULTRA-Sol detergent which has been accepted for use as aid in washing products in preparation for processing and preservation. Then, the washed tomato fruits were processed using sterilization, pasteurization, concentration by heating to make tomato puree. The same steps of home preparation and preservation methods were carried out for untreated washed tomato fruits to serve as control. This work was repeated three times by using 10 kg of tomato fruits for each experiment.

1.4. Extraction and purification

The lindane residues are extracted from treated tomato fruits (50 g sample) with 200 cm^3 acetone and the extract filtered. Then, 50 cm^3 of the filtration product diluted with 200 cm^3 of water and dichloromethane (1:1) for partition-

ing lindane residues. The extract was dried with anhydrous sodium sulfate and the dichloromethane layer was kept. The dichloromethane layer was removed by charcoal-silica gel purification column with the elution mixture (dichloromethane–benzene–acetone = 10:2:2). Then, the solvent mixture was evaporated and the lindane residues dissolved again in 10 cm^3 of n-hexane and diluted6-times in n-hexane for chromatographic determination, (TAG EL-DIN, 1987).

1.5. Recovery value of lindane

The efficiency of the gas liquid chromatographic method for residue determination of lindane was achieved by adding 100 μg lindane per g sample to a portion of untreated tomato sample which then was put through the extraction, purification and residual determination processes as followed in the used methods. Then, the recovery value was calculated as follows:

$$\text{Recovery value} = \frac{\mu\text{g lindane per g sample found}}{\mu\text{g lindane per g sample added}} \times 100$$

The average recovery value for lindane in tomato was 92.98%.

2. Results and discussion

2.1. Removal of lindane residues by a waiting period and washing

Removal of pesticide residues from plant foods by commercial washing controlled with many factors, such as chemical structure, chemical properties of the pesticide, nature of the food commodity and duration of the time that the compound has been in contact with the food, also the formulation in which the pesticide was applied and weather conditions as stated by ELKINS (1989). The effect of waiting period and home preparation are indicated in Table 1. The obtained data showed that the waiting period after lindane application and washing play a key role in reducing or removal of residue level on tomato fruits. A waiting period of 15 days post application led to a rapid removal and degradation of Lindane residues found on unwashed tomato samples ranging from 195.6 μg kg^{-1} to 148.8 μg kg^{-1} with a reduction percent of 23.9%. This finding coincides with those reported by FARROW and co-workers (1968; 1969) and BESSAR (1984).

Water washing reduced lindane residues immediately after application from 195.6 μg kg^{-1} to 133.6 μg kg^{-1} with a reduction percentage of 31.7%. Washing with detergent reduced the residues from 195.6 μg kg^{-1} to 112.4 μg kg^{-1} with a loss percentage of 42.6%. Fifteen days after treatment,

residues were reduced from 148.8 $\mu g\,kg^{-1}$ to 119.2 $\mu g\,kg^{-1}$ and to 65.7 $\mu g\,kg^{-1}$ as an effect of washing with tap water and with detergent. The trend as shown by the obtained data was in agreement with that of HEMPHILL and co-workers (1967); FARROW and co-workers (1968); LAMB and co-workers (1968); FAHEY and co-workers (1969), TALEKAR and co-workers (1977) and BESSAR (1984).

Table 1

Effect of simple and adequate washing and waiting period on lindane residues on tomato

Type of tomato washing	Lindane residues (μg per kg)						
	Immediately after treatment			15 days after treatment			Effect of waiting period
	Residues		Reduction	Residues		Reduction	$\frac{\text{15 days' residues}}{\text{Initial residues}} \times 100$
	$\bar{x}$	$\pm s$	(%)	$\bar{x}$	$\pm s$	(%)	
Unwashed "Initial"	195.6	6.3	00.0	148.8	7.2	00.0	76.1
Simple[a] washing	133.6	10.4	31.7	119.2	13.1	19.9	89.2
Adequate[b] washing	112.4	5.9	42.6	65.7	5.8	55.9	58.5

[a] Simple washing: washing with water
[b] Adequate washing: washing with detergent
The data are mean values and standard deviations of three parallels
Reduction percent = Initial residues — Found residues / Initial residues × 100

2.2. *Removal of lindane residues by the effect of some preservation processes*

As for the role of various technological processes in reducing the residue levels of lindane, data of lindane residues in tomato fresh juice, sterilized juice, pasteurized juice, concentrated juice and tomato puree are given in Table 2.

Table 2

Effect of various technological processes on lindane residues in tomato products

Technological processes	Lindane residues (μg per kg)						
	Immediately after treatment			15 days after treatment			Effect of waiting period
	Residues		Residue	Residues		Residue	$\frac{\text{15 days' residues}}{\text{Initial residues}} \times 100$
	$\bar{x}$	$\pm s$	(%)	$\bar{x}$	$\pm s$	(%)	
Fresh juice "Initial"	133.6	10.4	100	119.2	13.1	100	89.2
Sterilized juice	30.9	2.7	23.1	Non-detectable			00.0
Pasteurized juice	66.8	1.1	50.0	36.8	2.7	30.9	55.1
Concentrated juice	43.9	2.3	32.9	30.0	1.1	25.2	68.3
Tomato puree	26.6	2.3	19.9	20.9	1.1	17.5	78.6

The data are mean values and standard deviations of three parallel
Residue percent = Found residues / Initial residues × 100
Non-detectable: below the detectable limit (ng μl^{-1}) of the used gas-liquid chromatography instrument

Data showed clearly that pasteurization, concentration at high temperature, sterilization and sterilization of concentrated tomato juice and tomato puree decreased lindane residues by 50%, 32.9%, 23.1% and 19.9%, respectively, of the initial amount found in fresh tomato juice immediately after Lindane application. The same trend was found after 15 days subsequent to treatment, where residue levels were reduced to zero, 17.5%, 25.2% and 30.9% in sterilized juice, sterilized puree, concentrated juice and pasteurized juice, respectively. The prescribed waiting period of 15 days as a safety period between pesticid application and the time of marketing or consumption caused significant reduction in lindane residue levels as indicated in Tables 1 and 2. The obtained results strongly supported the view that, in spite of the high resistance of lindane to degradation by many physical factors, heat treatments, pasteurization and sterilization removed the major part of lindane residues adding to the effective role of washing as previously mentioned. The obtained data are in harmony with findings of KOIVISTOINEN and co-workers (1964); CARLIN and co-workers (1966); ELKINS and co-workers (1968); FARROW and co-workers (1969); TALEKAR and co-workers (1977); NEWSOME (1980); BESSAR, (1984) and ELKINS, (1989). In general waiting period for 15 days post treatment, washing and different technological processes in this investigation caused a higher reduction and removal of lindane residues to be within the established tolerance limits by U.S. ENVIRONMENTAL PROTECTION AGENCY (1978).

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FREE RADICAL REACTIONS IN MEATS

E. Dworschák[a], A. Lugasi[a], A. Blázovics[b], Gy. Biró[a], P. Biacs[c] and Á. J. Zsinka[c]

[a] National Institute of Food Hygiene and Nutrition, H-1037 Budapest, Gyáli út 3/a. Hungary
[b] Artheriosclerosis Research Group; Semmelweis Medical University, H-1085 Budapest, Somogyi B. u. 33. Hungary
[c] Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary

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Various methods were tested for the characterization of free radical formation and lipid peroxidation in meats. Among the intermediates of oxidative process malondialdehyde (MDA) and conjugated dienes were determined. Superoxide dismutase (SOD) and glutathione peroxidase (GSHpx) activities were evaluated as for information of the enzyme defence system. Direct and indirect chemiluminescence methods were also introduced for the characterization.

The formation of free radicals was monitored in meat samples with different fat content. The effect of repeated freezing and thawing was also studied. Each method applied can give special information on reaction involving oxidative processes. Chemiluminescence technique has a superiority because of its rapidity and its overall picture on the scavenger capacity and enzyme defence system.

Free radical formation was proportional with the fat content of samples. As membrane damage proceeds, the capacity of enzyme defence system decreased, but this was not valid for MDA and conjugated diene concentration.

Keywords: lipid peroxidation, membrane damage, antioxidant scavenger, enzymic defence, chemiluminescence

The formation of free radicals in the living organism is essential to maintain the normal physiological conditions. The functional balance is kept by defending mechanisms of chemical and enzymic origin. In unbalanced cases the excess of the reactive free radicals and their intermediates may damage the membrane structure. The changes of permeability in tissue will cause degenerative processes and later various diseases in the living organism, and in a similar way some detrimental changes in the quality of foods. It should be mentioned that these reactions not only impair the quality but they can play a role in the aethiology of various diseases in vivo (e.g. tumorogenic and theratogenic processes (Schamberger, 1980; Gryglewski, 1979).

Free radical reactions may decrease the quality of meats. These reactions may be initiated already in the living animals under the circumstances of improper feeding and breeding of animals (Dworschák & Prohászka, 1986). After slaughtering the conditions of storage and meat technology have a dominant role in the formation of free radicals. The lipid peroxidation in meats and meat products induce unfavourable changes in flavour and colour in the meats and even in the spices added, too.

According to our experiences the free radical reactions may contribute to the unsatisfactory quality of the carcase meats and meat products in Hungary and so far comprehensive studies have not been carried out in this area.

Our aim was to find suitable methods to characterize the changes caused by free radicals and lipid peroxidation and extrapolate them to the previous and subsequent states of the products. Only few data can be found on this topic in the literature.

1. Materials and methods

Beef and pork for analytical purpose was produced by the Meat Industrial Co, Budapest. Poultry liver samples were purchased on the markets in Budapest.

Meat biomembranes were damaged by freezing the meats to −18 °C and after a week defrosting them to room temperature. This procedure was repeated five times and the samples were taken and examined at the intervals.

The meat samples were mixed with tenfold amounts of buffer solutions and described in methods using a homogenizer (Thyristor, Janke and Kungel K. Gesellschaft). Homogenization took 5 minutes.

Two principles of the chemiluminescence technique were used: first the direct evaluation of free radicals; second: the defending enzyme (SOD and GSHpx) activities and the scavenger capacity of the meat samples (ZSINKA et al., 1988). The measurement was carried out with a CLD I Medicor-Medilab Luminometer (Hungary).

For the direct evaluation the luminol reagent solution consisted of 0.7 mmol l^{-1} luminol, 38 μmol l^{-1} hemin, 11.8 mmol l^{-1} Na_2CO_3 adjusted to pH 10 and deaerated with N_2. Reagent of 200–400 μl was added to the tissue homogenate (20 μl 1 mg cm^{-3} protein). The chemiluminescence intensity was expressed in mV, and it was recorded. Maximal intensity was 10 mV. The evaluation of a sample takes 60 s.

In the second case we measured the scavenging capacity of the superoxide radical evaluating the decrease of chemiluminescence intensity caused by the adrenaline–luminol reaction. The cuvette contained 20 μl tissue homogenate (1 mg cm^{-3} protein), 50 μl 10^{-3} mol l^{-1} adrenaline, 1000 μl luminol reagent and water up to the total volume of 1150 μl. Similarly the scavenging capacity of the peroxide radical was determined by the reaction between luminol and hydrogene peroxide. The cuvette contained 20 μl tissue homogenate (1 mg cm^{-3} protein), 50 μl 8.8 10^{-5} mol l^{-1} H_2O_2, 1000 μl luminol reagent and water up to the total volume of 1150 μl.

Malondialdehyde (MDA) was determined photometrically with the thiobarbituric acid reagent (OHKAWA et al., 1979).

Conjugated dienes were assayed by the absorbance at 233 nm (AOAC, 1984).

Among the enzymic defence, the activity of superoxide dismutase (SOD) was measured according to WINTERBOURN and co-workers (1975) on the basis of inhibition of adrenaline oxidation.

The activity of glutathione peroxidase (GSHpx) was evaluated by the photometric measurement of reduced glutathione after reacting with 5.5′-dithiobis (2-nitro-benzoic acid). (NOGUCHI et al., 1973).

Numerical data represent the average of four or five measurements. Standard deviations are also given in the text and in the figures, respectively.

For the statistical evaluation regression analysis was used where appropriate.

2. Results

Figure 1 shows the formation of free radicals characterized by the chemiluminescence intensity in chicken liver samples. The rate of formation of free radicals was proportional with the fat content of liver. The higher fat content in liver and membrane damage caused by freezing and the defrosting reduced the enzyme activities and the protective enzyme systems and the scavenger capacity in the tissues, and this is valid both for the adrenaline and hydrogen peroxide systems (Fig. 2).

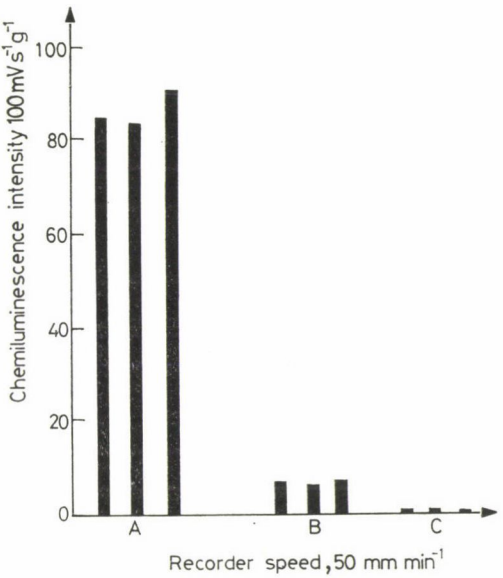

Fig. 1. Chemiluminescence intensity of free radicals in chicken livers (mV s^{-1} g^{-1}). Speed of recorder = 50 mm min^{-1}. Liver homogenate: A (fatty) = 12 g per 100 g fat; B (lightly fatty) = 8 g per 100 g fat; C (normal) = 2.5 g per 100 g fat

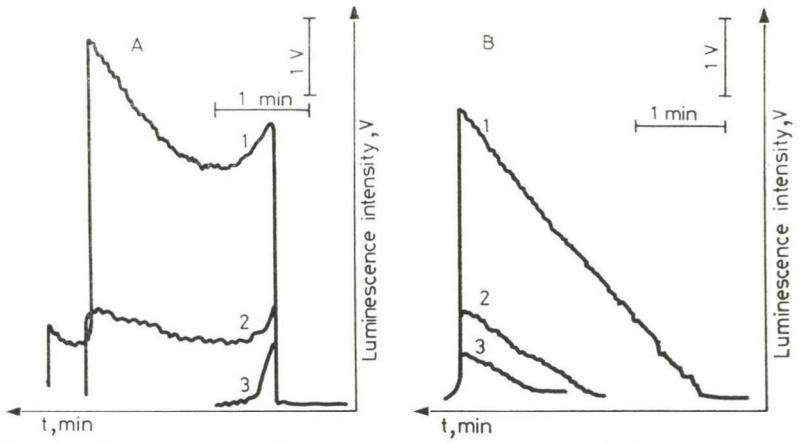

Fig. 2. Chemiluminescence intensity of the scavenger capacity in liver: A: scavenging of peroxide radicals; 1 = 30 μl 10^{-4} mol H_2O_2; 2 = +20 μl 3% goose-liver homogenate; 3 = +20 μl 3% ox-liver homogenate, B: scavenging of superoxide radicals; 1 = 50 μl 10^{-3} mol adrenaline; 2 = +20 μl 3% stored beef homogenate; 3 = +20 μl 3% fresh beef homogenate

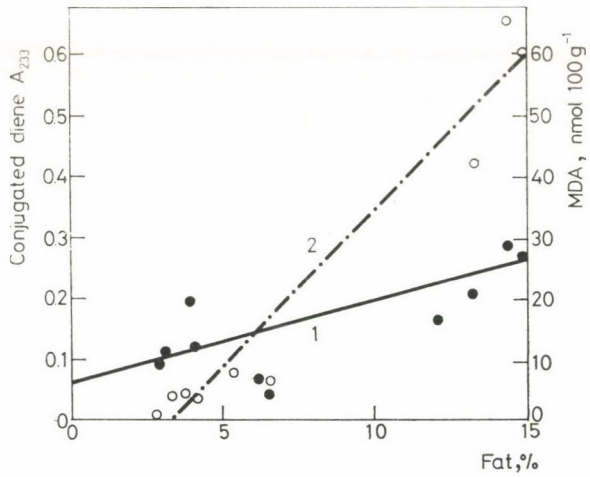

Fig. 3. Changes of MDA and conjugated diene levels in fresh meats as a function of fat content.. 1: MDA (y = 1.199x + 6.10; r = 0.74); 2: conjugated diene (y = 0.046x — 0.168; r = 0.90). The value of correlation coefficient (r) between MDA and conjugated dienes = = 0.84

In fresh meat samples MDA and the conjugated dienes gave a fairly good correlation with the fat content (Fig. 3). This means that the formation of free radicals and lipid peroxides starts already in fresh meat and its extent is proportional with the amount of fats.

The results of the fresh meat samples and after 3 consecutive deep-freezing and defrosting are introduced on Table 1. The level of MDA and conjugated dienes increased after membrane damage only in beef; in the fatty pork samples

Table 1

Effect of membrane damage on the enzymic defence and on the reactive intermediates of free radicals

	Beef (n = 5)				Fatty pork (n = 4)			
	before membrane damage		after membrane damage		before membrane damage		after membrane damage	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
MDA nmol per 100 g	8.75	3.1	11.4	1.0	22.9	5.6	25.4	9.7
Conjugated dienes A_{233} (1 g sample per 100 cm^3 isoctane)	0.041	0.02	0.107	0.04	0.452	0.23	0.437	0.37
SOD U per mg protein	2.12	1.0	1.95	0.2	2.38	0.8	1.99	0.4
GSHpx U per mg protein	0.95	0.5	0.70	0.3	0.76	0.3	0.62	0.2
Scavenging superoxide radicals $\frac{\text{mV sec } 10^6}{\text{1 mg protein}}$	1.96	0.4	1.95	0.2	1.85	0.2	1.58	0.2
Scavenging peroxide radicals $\frac{\text{mV sec } 10^6}{\text{1 mg protein}}$	1.95	0.3			1.86	0.2		

these values were originally high. The enzyme protection (SOD, GSHpx) was severely damaged after the intervention and the same is valid for the activity of scavenging superoxide radicals.

It was found that with the progress of membrane damage the MDA level gives a maximum indicating that MDA is not an end product of lipid-peroxidation (Fig. 4).

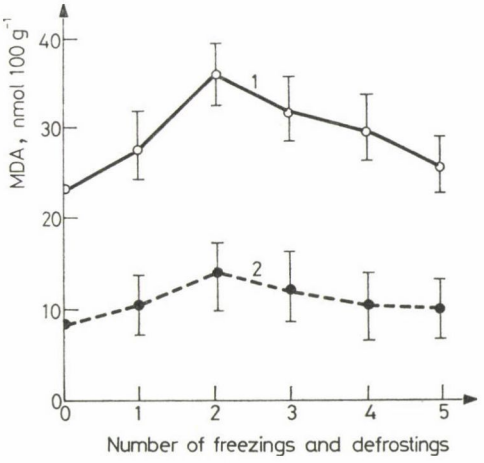

Fig. 4. Effect of membrane damage on the MDA level. 1: fatty pork; 2: beef

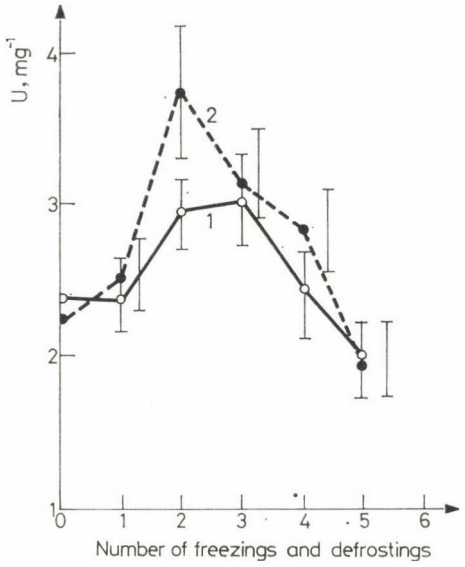

Fig. 5. Effect of membrane damage on the SOD activity. 1: beef; 2: fatty pork

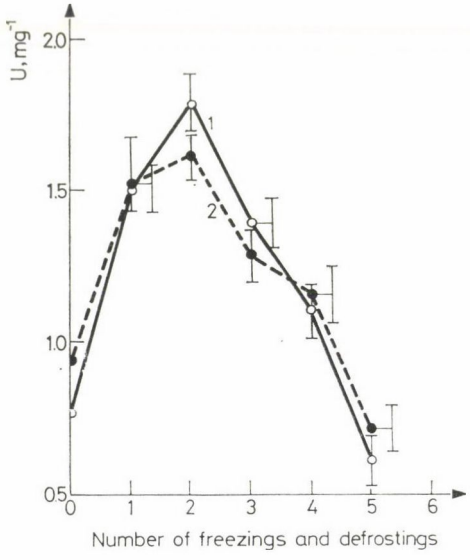

Fig. 6. Effect of membrane damage on the GSHpx activity. 1: fatty pork; 2: beef

In the first phase of membrane lesion activities of both SOD and GSHpx grew, probably because of the decompartmentalization. Further membrane damage could have exerted a denaturing effect on the enzymes to which the reactive intermediates of free radicals might have contributed with their destructive activity (Figs. 5 and 6).

3. Discussion

Because of the important role of free radicals and lipid peroxidation on meat quality, numerous methods have been developed to investigate these processes.

Malondialdehyde concentration (MDA) is generally accepted to characterize the extent of lipid peroxidation (BALUGIN et al., 1984) although some other reactions may disturb it (LOGANI & DAVIES, 1980). Because of the sensitivity of thiobarbituric acid reaction, MDA determination seems to prevail in food analysis. TICHIVANGAMA and MORISSEY (1985) ranged the sensitivity of meats of various animals using MDA determination: the rate of degradation was proportional to the amount of unsaturated fatty acids in meats from various animals.

Conjugated dienes proved to be also a reliable indicator for characterizing oxidation processes in fats (LOGANI & DAVIES, 1985). We found a better correlation between dienes and fat content than in case of MDA, the reason might be that dienes are formed at a later stage of oxidation processes.

SOD and GSHpx are the most important members of the enzymic defence system in living animals (XU & DIPLOCK, 1983, YOUNATHAN & MCWILLIAMS, 1985). Enzyme activities still remain after slaughtering the animals but there was a decrease in the enzymic defence with the degradation of membrane structure, as it was pointed out in our examination.

The above mentioned classical methods have different target points as regards the formation of free radicals in meats. However, none of them had a superiority both in quality and quantity of informations. Chemiluminescence technique has a wider acceptance now because of its advantages (YASUDA et al., 1986; ZSINKA et al., 1988). Direct detection of free radicals was carried out by us only in extreme circumstances (Fig. 1). Scavenging of superoxide and peroxide radicals has an overall information both for the scavenger compounds (antioxidants) and enzymic defence system (SOD, GSHpx). The most advantageous characteristic of the chemiluminescence technique is the rapidity: a sample can be analysed within one minute.

This work may be regarded as an orientating experiment for meat analysis. For the industrial practice the elaboration of new standardized methods and further experiments are needed.

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CHARACTERIZATION OF RAW AND DIELECTRIC HEATED SOYBEAN FLOURS OF TWO DIFFERENT PARTICLE SIZES

E. HORVÁTH and B. CZUKOR

Central Food Research Institute.
H-1022 Budapest, Herman Ottó út 15. Hungary

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Raw and dielectric treated soy samples were milled to full fat flours of particle size: 0–250 μm and 0–630 μm, respectively. Chemical composition, functional properties (water and fat absorption capacity, emulgation properties, nitrogen solubility index), protein extractability and in vitro protein digestibility of samples were determined. Results showed that the studied functional properties except for water absorption capacity and protein extractability of samples were decreased, in vitro protein digestibility was increased by dielectric heat treatment. The particle size of both raw dielectric treated samples affected their properties. Soy flours of smaller particle size (0–250 μm) had higher values of functional properties and higher protein digestibility and protein extractability in buffer Na_2HPO_4 (pH = 7.8) and in this buffer containing either sodiumdodecylsulfate (SDS) or SDS + 2-mercaptoethanol than soy flours of particle size 0–630 μm.

Keywords: heated soy flours, dielectric heating, protein digestibility

It is known that heating processes affect the quality of legume flours. There are several heating methods of legumes: steaming (NASH et al., 1979), extrusion cooking (MUSTAKAS et al., 1970), dielectric heating (BORCHERS et al., 1972), etc. Extrusion and steaming of soybeans have been examined in many studies but very little work has been done on the dielectric treatment of soybeans. It was stated that dielectric treatment inactivated the trypsin inhibitor and lipoxygenase, slightly increased water absorption capacity of soybeans (ESAKA et al., 1987; SAKLA et al., 1988).

The effect of particle size of flours upon their different properties has not been widely characterized yet. It was observed that the particle size distribution of air-classified flour samples was different and they could be characterized by different functional and other properties (SOSULSKI & YOUNGS, 1979). AGUILERA and GARCIA (1989) reported that the total extracted protein yield increased as particle size of lupin seeds decreased.

In an earlier work (HORVÁTH & CZUKOR, 1990) the effect of dielectric treatment on some properties of soybeans was studied, but the importance of the particle size was not taken into consideration. The objectives of the present study were to determine the functional properties, the protein ex-

tractability and in vitro protein digestibility of raw and dielectric heated (different from the earlier examination) soybean flours of particle size: 0–250 μm and 0–630 μm, respectively.

1. Materials and methods

1.1. Soy samples

Soybean seeds (*Glycine max*, variety: Ewans) were divided into two portions. One portion had the initial moisture content of 6.5% w/w and remained untreated. The moisture content of the other portion of soybeans was adjusted to 10% (w/w) by spraying prior to dielectric heating, and conditioned at room temperature for 24 h in an airproof polyethylene bag. Both the raw and dielectric heated whole soybean seeds were divided into two portions. One portion of each without dehulling was milled to full fat flour of particle size: 0–250 μm, and the other portion of each sample was milled to full fat flour of particle size: 0–630 μm placed in plastic bags and stored at 5 °C until required. When the whole soybeans were milled into fine flours of less than 250 μm particle size a very small proportion of the meal did not pass the 250 μm sieve and this crude, husky part was discarded.

1.2. Dielectric heating

Soybeans of 10% (w/w) moisture content were treated at a level of 27.12 MHz frequency in a Brown Boveri dielectric heater, in a completely filled wooden box (100×200×200 mm) between parallel plate electrodes, with an air gap of 5 mm. The anode voltage was 9.0 kV. The final temperature of soybean seeds was 120 °C. The temperature was measured with a toluene thermometer, placed into the geometric centre of the wooden box. Upon reaching the final temperature the heating was stopped and the soy samples were kept in the wooden boxes for 31 min. The soybeans were removed and permitted to cool in air at room temperature.

1.3. Analytical methods

1.3.1. Determination of moisture, protein and lipid contents. Crude protein (N×6.25) content was determined by the Kjeldahl method in an automatic Kjell–Foss equipment. Moisture content was measured by drying the samples to constant weight at 105 °C.

Lipid content was determined by petroleum ether extraction in Soxhlet apparatus.

1.3.2. Determination of functional properties. Water absorption capacity was analyzed by the method of Sosulski (1962), fat absorption capacity by

the method of LIN and co-workers (1974), emulsifying activity and emulsion stability by the method of YASUMATSU and co-workers (1972), Nitrogen Solubility Index (NSI) by the method of SMITH and CIRCLE (1972).

1.3.3. Extraction and fractionation of proteins. The fat free soy samples were extracted according to the method of OSBORNE (1924) and the method of THAN and SHIBASAKI (1976). Soy samples were also extracted with 0.5 mol dm^{-3} Na_2HPO_4 (pH = 7.8) buffer, and with the same buffer containing either 5% (w/v) sodiumdodecyl sulphate (SDS) or 5% (w/v) SDS and 1% (v/v) 2-mercaptoethanol (2-ME) by the method of HORVÁTH and CZUKOR (1988).

1.3.4. Determination of in vitro protein digestibility. In vitro protein digestibility was measured by the method of AKESON and STAHMAN (1964).

2. Results and discussion

2.1. Moisture, protein and lipid content

Table 1 shows the moisture, protein and lipid contents of raw and dielectric heated soybeans milled without dehulling to full fat flours of particle size 0–250 μm and 0–630 μm, respectively.

The moisture content of treated soy samples was lower than the moisture content of raw samples. The particle size of flours did not affect the moisture content. The raw protein (N×6.25) and lipid contents of samples are referred to dry matter. We have found that the lipid and protein contents of the soy

Table 1

Moisture, lipid, and protein (N × 6.25) content of raw and dielectric heated soy flours of different particle size

Sample	Moisture content (g per 100 g total weight)		Lipid content (g per 100 g dry weight)		Protein (N×6.25) content (g per 100 g dry weight)	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
1. Raw particle size 0–250 μm	6.4	0.2	22.0	0.3	41.5	0.2
2. Dielectric heated particle size 0–250 μm	4.8	0.1	21.6	0.5	41.6	0.1
3. Raw particle size 0–630 μm	6.5	0.1	19.7	0.5	40.8	0.1
4. Dielectric heated particle size 0–630 μm	4.9	0.1	20.6	0.4	40.6	0.1

$\bar{x}$: mean value of three mesurements
±s: standard deviation

samples of smaller particle size were higher than those of larger particle size. This can be explained by the fact that we had difficulties to mill the whole soybeans into fine flours of less than 250 μm particle size. A small part of the soy meal could not be passed through the 250 μm sieve and this crude, husky part was discarded. This might cause the higher protein and lipid content of the finer flours.

2.2. *Functional properties*

Functional properties of soy samples are shown in Table 2.

Table 2

Functional properties of raw and dielectric heated soy flours of different particle size

Sample	Water absorpcion capacity (g per 100 g sample)		Fat absorption capacity (g per 100 g sample)		Emulsifying index (% v/v)		Emulsion stability (%, v/v)		Nitrogen solubility index (NSI) (%, w/w)	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
1. Raw particle size 0–250 μm	159.4	0.8	140.9	0.8	72.7	1.8	76.3	0.3	75.0	0.4
2. Dielectric heated particle size 0–250 μm	129.3	2.3	119.6	6.9	59.0	0.8	57.0	0.2	8.4	0.3
3. Raw particle size 0–630 μm	176.6	3.5	125.7	4.7	68.2	0.6	67.9	1.2	50.6	1.1
4. Dielectric heated particle size 0–630 μm	185.8	1.1	114.1	1.3	56.0	0.6	50.2	1.0	6.4	0.2

$\bar{x}$: mean value of three measurements
$\pm s$: standard deviation

Both raw and dielectric treated soy flours of larger particle size (0–630 μm) have higher water absorption capacity (WAC) than fine flours (0–250 μm). Data reveal that the effect of the particle size on WAC is important, and the effect of dielectric treatment on WAC is not unambiguous.

Comparing the fat absorption capacity (FAC), emulsifying activity (EA), emulsion stability (ES) and nitrogen solubility index (NSI) of raw and dielectric heated soy flours of particle size 0–250 μm with soy flours of particle size 0–630 μm it can be seen that the smaller particle size resulted in higher FAC, EA, ES and NSI values. Dielectric treatment affected also the functional properties. It was found that dielectric heated soy samples had lower FAC EA, ES and NSI values than the raw samples having the same particle size.

These results suggest that not only heat denaturation and changes in protein structure caused by dielectric treatment (HORVÁTH & CZUKOR, 1990) but the particle size of soybean flours also affects the functional properties

2.3. Extraction and fractionation of soy proteins

The percentage of H_2O-, NaCl-, C_2H_5OH-, and KOH- soluble protein fractions was determined according to the Osborne method. Results are presented in Table 3.

Soy proteins were fractionated by the method of THAN and SHIBASAKI (1976) as well, extracting 7S, 11S and whey soy proteins. The results are presented in Table 4.

It is known that dielectric heat treatment significantly decreases the extractability of proteins from soybeans (HORVÁTH & CZUKOR, 1990) denoting denaturation of soy proteins. These results confirm those data. The proportions of H_2O- and NaCl-soluble and 7S and 11S fractions in total protein decreased significantly.

The particle size of soy flours affected the protein extractability of raw samples. In these examinations from dielectric heated soy samples of different particle size nearly the same percentage of proteins could be extracted by both methods denoting that after dielectric treatment changes in protein structure took a prominent part and using this extraction method the effect of particle size was not important.

Table 3

Extraction yield of proteins from raw and dielectric heated soy flours of different particle size by Osborne method

Sample	Extracted proteins in total protein (%; w/w)		Percentage of fraction in total protein							
			H_2O-		NaCl-		C_2H_5OH-		KOH-	
			soluble proteins							
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
1. Raw particle size 0–250 μm	53.7	3.3	24.5	3.4	23.3	0.3	1.4	0.2	4.5	0.1
2. Dielectric heated particle size 0–250 μm	8.9	0.2	4.2	0.2	2.6	0.1	0.8	0.0	1.2	0.0
3. Raw particle size 0–630 μm	41.7	1.7	17.8	0.6	18.7	0.3	2.4	0.2	2.9	1.3
4. Dielectric heated particle size 0–630 μm	9.5	1.8	4.1	0.9	2.8	0.9	1.0	0.2	1.7	0.4

$\bar{x}$: mean value of four measurements
$\pm s$: standard deviation

Table 4

Extraction yield of proteins from raw and dielectric heated soy flours of different particle size by the method of Than and Shibasaki

Sample	Extracted proteins in total protein (%; w/w)		Percentage of fractions in total protein					
			7S		11S		Whey protein	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
1. Raw particle size 0–250 μm	56.4	1.8	25.0	1.1	13.3	1.2	18.0	0.4
2. Dielectric heated particle size 0–250 μm	18.5	0.6	5.5	0.1	2.1	0.1	10.9	0.6
3. Raw particle size 0–630 μm	49.1	0.6	23.5	2.1	9.9	1.5	15.7	0.9
4. Dielectric heated particle size 0–630 μm	17.5	0.6	5.4	0.1	2.0	0.1	10.1	0.7

$\bar{x}$: mean vaue of four measurements
±s: standard deviation

Table 5

Extraction yield of proteins from raw and dielectric heated soy flours of different particle size in 0.5 mol dm^{-3} Na_2HPO_4 solution (pH = 7.8) and in 0.5 mol dm^{-3} Na_2HPO_4 solution (pH = 7.8) containing either 5% (w/w) SDS or 5% (w/w) SDS and 1% (v/v) 2-ME

Sample	Extracted proteins in total protein (%; w/w)					
	Extraction solution					
	0.5 mol dm^{-3} Na_2HPO_4 (pH = 7.8)		0.5 mol dm^{-3} Na_2HPO_4+5% SDS (pH = 7.8)		0.5 mol dm^{-3} Na_2HPO_4+5% SDS+1% 2-ME (pH = 7.8)	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
1. Raw particle size 0–250 μm	69.4	5.2	54.1	3.4	48.8	1.6
2. Dielectric heated particle size 0–250 μm	8.8	0.4	21.9	0.7	36.9	2.1
3. Raw particle size 0–630 μm	51.2	1.0	41.8	2.8	40.0	1.1
4. Dielectric heated particle size 0–630 μm	8.4	2.7	12.4	1.2	25.8	0.4

$\bar{x}$: mean value of four measurements
±s: standard deviation

Solubility of soy proteins was determined by using 0.5 mol dm^{-3} Na_2HPO_5 (pH = 7.8) buffer and the same buffer containing either SDS or SDS + 2–ME. The results are shown in Table 5.

After dielectric treatment proteins lost most of their solubility in the buffer. The SDS is an agent known to disrupt non-covalent interactions and 2–ME is an agent known to reduce disulfide bonds (JEUNINK & CHEFTEL,

1979; CLATTERBUCK et al., 1980). Solubilizing activity of the buffer containing SDS or 2-ME in heat treated soy samples was found higher than that of the plain buffer, suggesting that dielectric heat treatment increased disulfide and hydrogen, and/or hydrophobic bonds in soy flours.

Particle size of both raw and dielectric treated samples affected also the protein solubility in different solvents. Except for the protein solubility of dielectric heated samples in 0.5 mol dm^{-3} Na_2HPO_4 (pH = 7.8) soy samples of smaller particle size (0–250 μm) showed higher protein solubility in all extraction solutions than samples of larger particle size (0–630 μm).

Table 6

In vitro protein digestibility of raw and dielectric heated soy flours having different particle size

Sample	In vitro protein digestibility (%)	
	$\bar{x}$	$\pm s$
1. Raw particle size 0–250 μm	78.2	4.0
2. Dielectric heated particle size 0–250 μm	85.5	2.3
3. Raw particle size 0–630 μm	76.6	3.5
4. Dielectric heated particle size 0–630 μm	82.8	2.8

$\bar{x}$: mean value of three measurements
$\pm s$: standard deviation

2.4. In vitro protein digestibility

In vitro protein digestibility of soy samples can be seen in Table 6.

Remarkable differences were not found between the in vitro protein digestibility of raw and dielectric treated soy samples and between the soy samples of different particle size. But there is a tendency that dielectric treatment increased the protein digestibility of soy meal and the samples of smaller particle size had higher digestibility.

3. Conclusions

Summarizing the results it can be concluded that these experiments confirmed the substantial effect of dielectric heat treatment on the properties of soybean. The very important finding is that the particle size of flour samples (both raw and dielectric treated) affected substantially the different functional

properties, protein extractability and protein digestibility of soy samples. The results proved that it was absolutely necessary to determine the particle size of soy flour samples to be able to compare their characteristics and by decreasing the particle size higher values of properties could be achieved. It is worth milling the flours into small particle size because they can be better used in food technology processes.

*

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INFLUENCE OF THE STATE OF RIPENESS OF CHARDONNAY GRAPES ON WINE COMPOSITION. I. PHYSICOCHEMICAL CHARACTERISTICS, HIGHER ALCOHOLS, POLYOLS AND ESTERS

M. P. CALLAO[a], J. M. BORRAS[a], A. LOPEZ[b] and F. X. RIUS[a]

[a] Departament de Quimica. Facultat de Ciéncies Quimiques de Tarragona. Universitat de Barcelona, 43005 Tarrogona. Spain
[b] Departament de Tecnologia d'Aliments. E. T. S. E. A. Universitat Politécnica de Catalunya, 25006 Lleida. Spain

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The present study illustrates the most significant physico-chemical characteristics, as well as the composition of majority alcohols, two polyols and twelve esters in wines made from must of Chardonnay grapes, corresponding to different states of ripeness. Wines with a remarkable difference in alcoholic strength do not show significant differences with regard to superior alcohols or esters, except for 2-phenyl-ethanol acetate and cis-3-hexenyl acetate. Grapes with total acidity levels of 9.8 g l^{-1} and 20 °Brix lead to the production of wines with acceptable flavour and with an alcoholic strength of 10.5 degrees.

Keywords: wine characteristic, Chardonnay grapes, ripeness of grapes

Nowadays, the trends of white wine consumption point to light, low alcohol content, young, fruity and somewhat acid wines. However, for the production of low alcoholic degree white wines it is essential that the departure musts have a relatively low level of sugars. Notwithstanding this, the use of unripe grape harvest may affect the organoleptic characteristics of the wine in a negative way (BERTRAND, 1980; LOPEZ, 1985).

Therefore, it seemed necessary to carry out some studies in order to determine the most suitable level of grape ripeness for each variety, so as to establish a balance between relatively low alcohol content and the good taste of the white wine produced (VAN ROOYEN et al., 1984).

A number of studies in this line of research have already been published, the aim of which has been to find out the best ripeness level of the grapes, in order to obtain the best quality wines (DU PLESSIS, 1983; ELLIS et al., 1985; IANINI et al., 1985; VAN ROOYEN et al., 1984). More specifically, research has been carried out on wines produced from Chardonnay grapes paying special attention to the maceration effects (ARNOLD & NOBLE, 1979; RAMEY et al., 1986; TEST et al., 1986), the fermentation techniques (COTTRELL & MCLELLAN, 1986), soil composition (NOBLE, 1979). However, it seems there are not enough studies that try to determine the optimum ripeness state of this grape variety.

For this reason, a number of studies will be published, being this the first one of the series, in order to propose an explanation which could allow the establishment of the most suitable ripeness level of the Chardonnay type grapes, regarding the composition of the white wine produced from this variety. This composition will be evaluated according to its physicochemical characteristics and volatile substances content.

1. Materials and methods

1.1. Raw materials and production process

Experiments have been done on grapes of the Chardonnay variety, harvested on three different dates: August 28, 1986; September 1, 1986; September 8, 1986, from Raymat vineyards (Lleida, Spain).

After removing stems, grapes were gently pressed and must was obtained on an endless band press of 60% yield. Sulphite was added at the rate of 30 mg l^{-1}. Solid residues were removed using a vacuum rotary drum filter. Must obtained in this way, at industrial rate, was then fermented in pilot plant in stainless steel containers of 75 l capacity at 18 °C temperature. Before the beginning of fermentation, 20 g 100 l^{-1} of bentonite and 10 g 100 l^{-1} of selected yeast (*Saccharomices cerevisiae*) were added to the must.

1.2. Analytical methods

Must samples were taken inmediately before adding yeast and bentonite, those of wine-must during alcoholic fermentation and those of wine at the end of fermentation.

Fermentation was carried out according to three states of grape ripeness, with repetitions for each one of these states of ripeness.

The physicochemical characteristics of must and wines were determined following official methods of analysis; determination of glycerol in wines was carried out by enzymatic analysis and spectrophotometry, and 2,3-butanediol was determined by gas chromatography, with direct injection in a Tenax column (Casp et al., 1985). Methanol and higher alcohols except for 2-phenyl-ethanol were determined by gas chromatography, using the internal standard technique with a 4 m $\times$ 2.15 cm column, type Carbowax 1500 at 15% on Chromosorb W (Casp et al., 1985).

The remaining components studied (12 esters), all of them minority components, were determined by gas chromatography after previous extraction of the samples with organic solvents.

Following the extraction method described by DI STEFANO (1985), two successive wine extractions were carried out, the first one with pentane for six hours, and the second one using a mixture of pentane and dichloromethane (3 : 2) for eight hours with an extraction coefficient near to 100%. Neither of these solvents is very selective, an essential quality when it is necessary to obtain information about the maximum number of components. Once each extraction was finished, the organic phases were gathered after drying them up with anhydrous sodium sulfate in a heart-shaped flask and were concentrated up to 0.5 cm^3 approximately, using a Vigreux column. Regarding the quantitative analysis, methyl caprylate was used as internal standard.

The equipment used was a Hewlett Packard (HP) model 5890 gas chromatograph, fitted with a flow split injector, flame ionization detector, cnd coupled to an HP 300 personal computer with monitor and printer inaorporated.

The column used was a chemically linked phase capillary column, type Carbowax 20M of 50 m $\times$ 0.2 mm and 0.25 μm thickness. Number of effective plates: 2377 m^{-1}.

2. Results and discussion

The physicochemical characteristics of departure must are those shown in Table 1.

Fermentation of each must occured according to data in Fig. 1, showing that fermentation was faster and more complete in must from riper grapes.

The obtained wines had the physicochemical characteristics shown in Table 2, which illustrates that the alcoholic content of wines varied between 10.55 and 12.17 (i.e., 15.35%) in the harvest period from August 28 to Sep-

Table 1

Physicochemical characteristics of departure musts

	Vintage					
	28–8–1986		1–9–1986		8–9–1986	
	Measured values					
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Density at 20 °C	1.0825	0.0006	1.0865	0.0006	1.0915	0.0006
°Brix	20.10	0.16	20.42	0.12	21.30	0.35
pH	3.13	0.02	3.07	0.03	3.15	0.02
Titratable acidity (g l^{-1} tartaric acid)	9.86	0.12	9.60	0.06	8.16	0.11
Suspended solids (g l^{-1})	0.658	0.071	0.718	0.051	0.712	0.051
Sugars (g l^{-1})	194	1.6	198	1.2	207	3.5

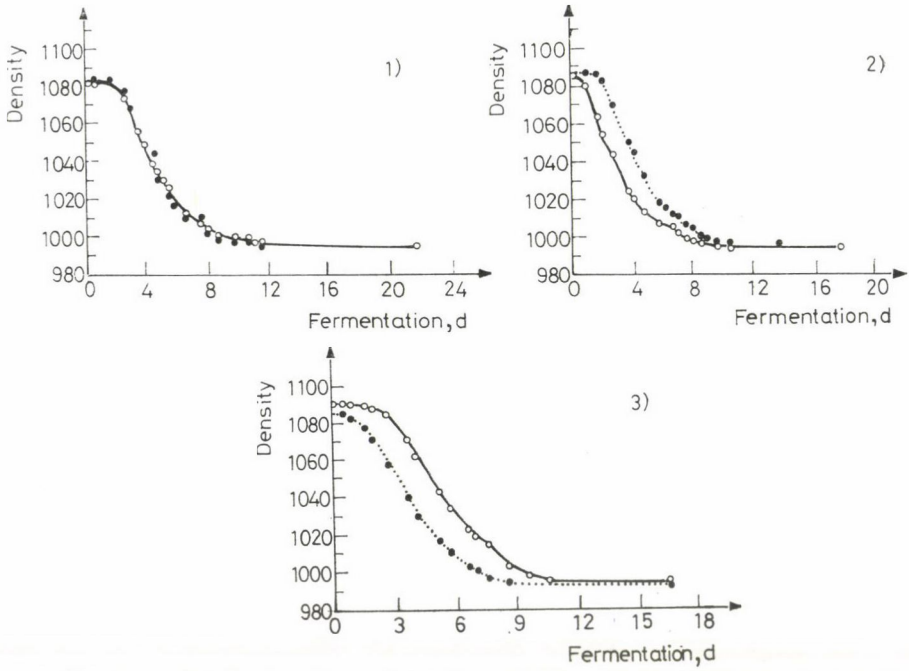

Fig. 1. Evolution of density throughout fermentation. Graph 1: lower ripeness level; graph 2: indermediate ripeness level; graph 3: higher ripeness level. Deposit 1: o; deposit 2: ●

tember 8, 1986. Total acidity was also significantly reduced with increasing grape ripeness.

Table 3, showing methanol, higher alcohols and polyols, illustrates that the content variation of polyols, caused by the different states of grape ripeness, is significant but very low. Something similar occurs regarding l-propanol, isobutanol, and isoamyl alcohols. To a certain extent, the most important variation can be observed in methanol and it was caused by the higher or lower level of unripeness of the grapes.

Figure 2 illustrates the chromatograms obtained with both a pentane extract and a pentane-dichloromethane extract of a wine sample studied. The analyzed esters are shown in each chromatogram with a number that corresponds to the number allocated to each component in the legends of the figures.

The ester average contents in the obtained wines and their analysis of variance is shown on Table 4. The evolution of each ester development during fermentation, according to the ripeness state of the harvest is shown in three-dimensional graphs (Fig. 3). Again, the numbers allocated correspond to those displayed in the chromatograms.

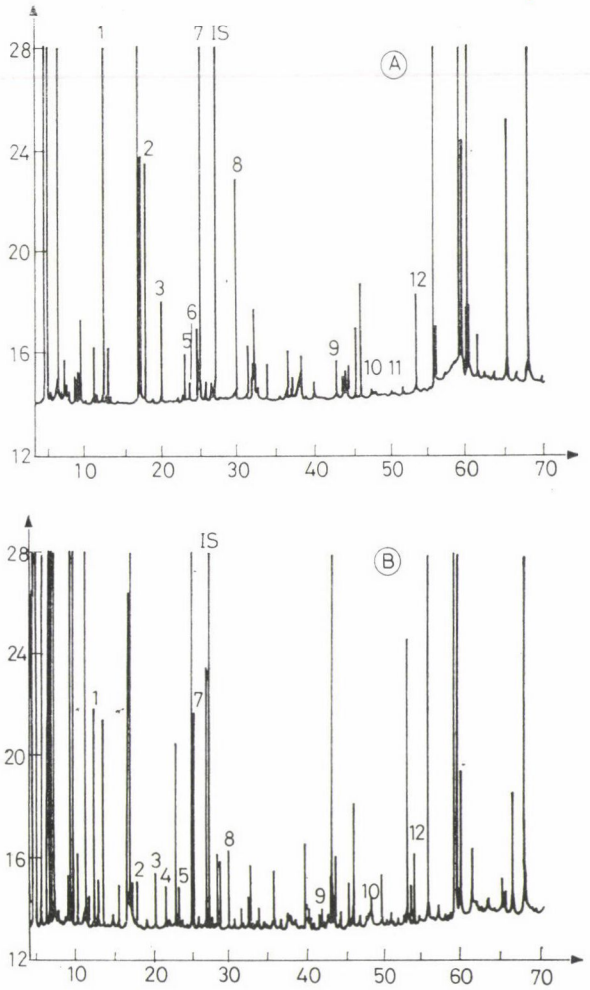

Fig. 2. Chromatograms of a wine extract. A) in pentane. B) in pentane-dichloromethane. Chromatographic conditions: gas flow: carrier gas: He = 0.68 cm^3 min^{-1}, air: 326 cm^3 min^{-1}, H_2 = 32 cm min^{-1}; auxiliary gas, He = 70 cm^3 min^{-1}, division ratio = 1:70; working temperatures: injector = 250 °C; detector = 250 °C; temperature programme: initial = 60 °C (2 min), ramp(R) = 2 °C min^{-1}, final = 180 °C (20 min). IS: Internal standard (methyl caprylate); 1: isoamyl acetate; 2: ethyl caproate; 3: hexyl acetate 4: ethyl piruvate; 5: cis-3-hexenyl acetate; 6: ethyl heptanoate; 7: ethyl lactate; 8: ethyl caprilate; 9: ethyl caprate; 10: ethyl undecanoate; 11: dietyl glutarate; 12: 2-phenylethyl acetate

Each graph shows how the corresponding ester concentration increases throughout the fermentation process, and decreases at the final stage of fermentation. Moreover, it is also significant to note in these plots that the variations between the maximum quantities, measured in mg l^{-1} of ester, formed during fermentation are not very important, especially regarding ethyl

Table 2

Physicochemical characteristics of wines

	Vintage					
	28–8–1986		1–9–1986		8–9–1986	
	Measured values					
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Density at 20 °C	0.9960	0.0000	0.9950	0.0000	0.9930	0.000
pH	3.04	0.02	2.96	0.06	3.08	0.01
Alcoholic degree at 20 °C	10.55	0.10	11.42	0.12	12.18	0.05
Titratable acidity (g l^{-1} tartaric acid)	10.18	0.13	9.47	0.04	8.08	0.20
Volatile acidity (g l^{-1} acetic acid)	0.28	0.04	0.25	0.02	0.21	0.02
Reducing sugars (g l^{-1})	1.48	0.10	1.58	0.05	1.22	0.10
Total sulfur dioxide (mg SO_2 l^{-1})	48.0	3.7	22.4	0.0	28.8	0.0
Free sulfur dioxide (<3 mg SO_2 l^{-1})	<3	—	<3	—	<3	—
Optical density at 420 nm	0.119	0.006	0.116	0.007	0.113	0.006
Total polyphenol index	9.625	0.096	9.562	0.048	8.875	0.050
Tannin (g l^{-1})	0.67	0.01	0.67	0.00	0.62	0.00
Ash content (g l^{-1})	2.13	0.09	2.83	0.22	2.32	0.13
Ash alkalinity (meq l^{-1})	47.1	0.2	47.8	0.3	47.0	0.1

Table 3

Methanol, main higher alcohols and polyols in wines

	Vintage					
	28–9–1986		1–9–1986		8–9–1986	
	Measured values					
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Methyl alcohol (mg l^{-1})	67	4	60	6	37	3
1-Propyl alcohol (mg l^{-1})	39	4	39	3	48	6
Isobutyl alcohol (mg l^{-1})	34	4	32	2	43	6
Isoamyl alcohols (mg l^{-1})	99	6	103	4	123	6
2,3-Butandiol (g l^{-1})	0.16	0.02	0.17	0.01	0.28	0.02
Glycerol (g l^{-1})	3.15	0.17	3.28	0.03	3.58	0.02

Table 4

Esters in wines

(mg l^{-1})

	Vintage			Analysis of variance	
	28-9-1986	1-9-1986	8-9-1986	F ratio	sig level
Isoamyl acetate	1.796	1.446	2.673	1.012	0.4614
Ethyl caproate	0.414	0.404	0.478	8.554	0.0576
Hexyl acetate	0.136	0.148	0.148	0.014	0.9857
Ethyl piruvate	0.541	0.669	0.682	3.294	0.175
cis-3-hexenyl acetate	1.263	0.020	0.030	38.720	0.0072
Ethyl heptanoate	0.028	0.014	0.061	0.679	0.5711
Ethyl lactate	4.124	4.928	4.200	3.776	0.1516
Ethyl caprilate	0.460	0.448	0.478	0.412	0.6947
Ethyl caprate	0.170	0.090	0.158	4.367	0.1293
Ethyl undecanoate	—	0.025	—	—	—
Diethyl glutarate	0.114	0.104	0.086	0.997	0.4656
2-Phenylethyl acetate	0.091	0.078	0.219	10.333	0.0451

caprylate, ethyl lactate, hexyl acetate, ethyl caproate, isoamyl acetate, diethyl glutarate, ethyl caprate and ethyl undecanoate.

The graphs also illustrate that there is a decrease in the content of cis-3--hexenyl acetate in wine as the state of grape ripeness increases, whereas the rates of 2-phenyl-ethanol acetate show an opposite evolution.

Regarding ethylheptanoate, a clear trend evolution could not be observed perhaps due to the low level of this component in wine.

Although a clear trend of the influence of the state of ripeness on wine composition can be observed, definitive conclusions cannot be reached as in the present work only 12 days of the grape harvested in a year has been studied.

3. Summary

The above mentioned analytical results showed that the differences in alcohol content obtained in the wine samples studied were very important. However, there were no remarkable differences in the rates of higher alcohols, glycerol, 2,3-butanediol or esters, except for 2-phenylethanol acetate and ethyl pyruvate.

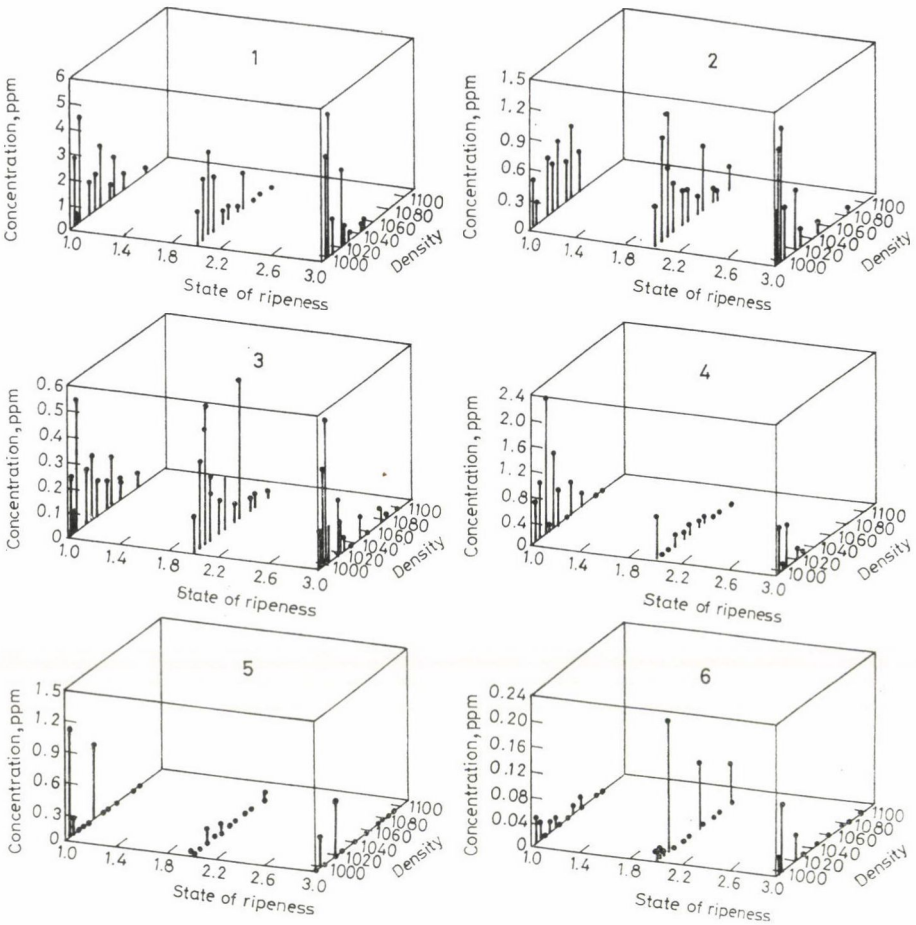

Fig. 3. Evolution of ester content throughout fermentation process for

Summing up, from Chardonnay grapes harvested in the above mentioned Spanish area, with ripeness of about 200 Brix and total acidity of 9.8 g l^{-1} of tartaric acid, an acceptable wine quality can be obtained, regarding flavour and ester content, and with a relatively low alcoholic grade of 10.5, which matches the market trends for quality white wines.

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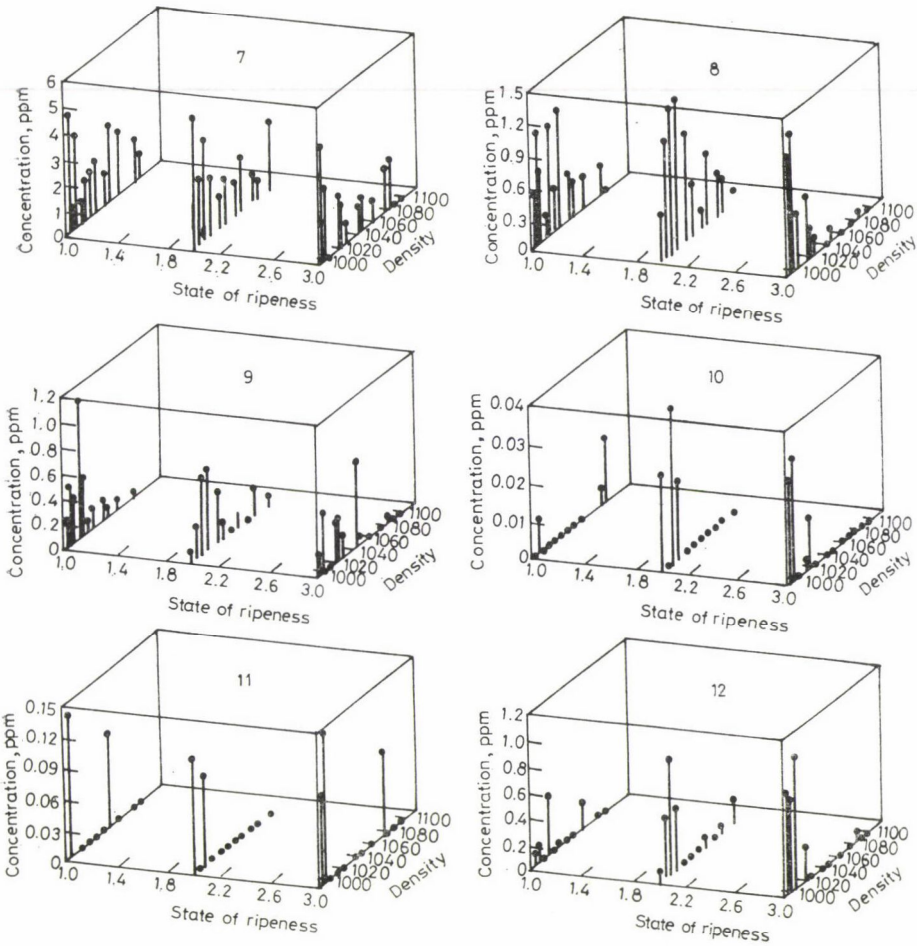

each ripeness level. The esters concentration in wines is related in Table 4

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ABSTRACTS

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RESULTS OF TESTING SENSORY EVALUATION METHODS

P. MOLNÁR, V. NÁGEL and L. KATONA

Institute of Food Control, H-1095 Budapest, Mester u. 81. Hungary

At present there are many different scoring methods of sensory evaluation used in the Hungarian food quality control. Looking back on the last few years it can be seen that the 20 point scoring system including weighting factor based on general impression gains ground in more and more branches of the industry over more and more product groups. In the frame of the present research program — in the interest of unification — this evaluation system was developed for 200 products in 13 branches of the food industry. Parallel to the development some of the methods were tested. In the course of testing the current standard method was used as the basis for comparsion e.g. in the canning and refrigeration industries the 100 point scoring system, in the distilling industry the traditional 20 point scoring system and in the meat industry the 20 point product describing system. Testing was carried out as the joint effort of experts from industry and authority. In the course of evaluating the results:

— the formation of the total scores (by three-point analysis of variance) and the differences in methods were analyzed;

— "the product sensitivity" was characterized by the standard deviation of total scores;

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— the safety of method application was characterized by the standard deviation among the scores given by individual judges;

— the applicability of the methods was evaluated by the extent of the given scores;

— the independence of characteristics was established by the analysis of the main components while interdependence was found between scores given to flavour and smell;

— no significant difference was found between the scores given by experts from industry and authority, therefore it was concluded that where in daily evaluations the differences are permanent the experts are affected in their judgement by external influence;

— the correlation between methods was studied by regression analysis thereby evaluating the changes of introducing the new methods.

UNIVERSAL LABORATORY TEXTUROMETER

Á. Álmos

Research Institute for the Canning Industry,
H-1097 Budapest, Földváry u. 4. Hungary

Importance of developing and universal laboratory texturometer: necessity of rheological measurements. Investigation of the deformation of agricultural raw materials and products of the food industry caused by the application of force serves as a means to determine the quality or ripeness of the produce, etc. Simple manual measuring instruments:

— measurement of deformation caused by a constant force;

— measurement of the force causing a given deformation (e.g. manual penetrometer);

— Finometer, an instrument for the measurement of tenderness in young peas, operated by a crank arm through transmission;

— measuring device recording maximum force by manual digital signal; Electric motor operated device recording maximum force. Instruments suitable for plotting loading force — characteristic curve.

— Main parts: loading unit, metering-sensing unit, recording unit.

— Evaluation of the recorded curve: shape of the curve, characteristic points.

Evaluation of the electrically operated Finometer, developed in the Research Institute for the Canning Industry.

Fresh corn testing instrument developed in the Research Institute for the Canning Industry.

Results of the development of the universal laboratory texturometer constructed in the Research Institute for the Canning Industry.

The texturometer is based on the sweet corn testing instrument. The texturometer is suitable for carrying out different measurements. Some of its parameters have been made adjustable. The maximum pressing force, the speed and the path covered of the instrument sensoric head can be set. The force measuring units of necessary measuring limits are changeable and so are the various pressing, piercing and cutting accessories in touch with the material to be tested. A recorder can be attached to plot the curve of the loading force and the deformation.

A further aim of the Institute is to develop a simple, cheap texturometer family embracing in several steps the whole domain of force occurring in the practice.

APPLICATION OF PAS TECHNIQUE IN FOOD ANALYSIS

L. Kocsányi[a], P. Richter[a], F. Mohos[b], M. Váradi[b] and P. Biacs[b]

Department of Atomic Physics, Technical University, Budapest, H-1111 Budafoki út 8. Hungary
[b]*Central Food Research Institute, H-1022 Budapest, Herman O. út 15. Hungary*

The aim of photothermic and within it photoacoustic investigations was to determine heat development in the course of light absorption. In spectroscopic analyses an amount in proportion with rising temperature is measured as a function of the wavelength of the exciting light. There are several suitable photothermic detectors known of which the most widely accepted and most sensitive is the photoacoustic cell.

Two such photothermic detectors and their application is described. One of them is the photoacoustic cell with a closed sample space while the other one is the photopyroelectric sensor developed by the authors. The investigations fall into two groups:

1. Recording of spectra in the NIR domain (800–2200 nm) of powders (powdered dyes, starch, flour) and of liquids (milk). Comparison with reflection spectra.

PAS technique proved to be suitable for the absorption analysis of the above samples. Its advantage over the reflection investigations lies in the fact that even in the presence of very strong absorbants (e.g. water in milk) it is possible to record a saturation-free spectrum. Its disadvantage lies in the time requiring measurement and the light requirement of exposure.

2. Photopyroelectric and photoacoustic investigations in the visible domain of powdered dyes and plant leaves.

The spectra obtained by the photopyroelectric sensor are identical with those obtained with the photoacoustic cell. The advantage of the sensor over

the cell lies in the fact that the sample is not enclosed in the sample space. The measurement is carried out simply by putting the sensor in touch with the sample.

Apart from sensing being more simple the method can be used to the photothermic analyses of samples where photochemical reactions occur accompanied by gas development (e.g. photosynthesis in leaves). The photoacoustic cell is not suitable for the analysis of such samples because of its sensitivity to gas development in its closed sample volume.

SIMULTANEOUS DETERMINATION OF DIFFERENT MEAT PROTEINS

W. Mezei-Dudonis[a] and P. Györei[b]

[a] *Central Laboratory for Quality Control of the Hungarian Meat Industry, H-1095 Budapest, Soroksári út 58. Hungary*
[b] *Institute for Public Health and Epidemiology of County Pest, H-1032 Budapest, Váradi Sándor u. 35/41. Hungary*

A short description of the results achieved in the frame of the research theme under the title "Development of quality-centered standard aimed at the control of quality in the meat industry", is given. Results of the subsubject: "Protein determination method developed to simultaneously separate the meat of different animals" is discussed in detail.

In quality control increasing importance is attached to the quality of meat and within this question the animal species processed. Beginning with January 1990 the USDA specifications require to carry out the "species identification" test in all animal products manufactured for export. On the basis of data in the literature and the results of the preliminary experiments, apart from the ELISA technique, out of the gel-electroforetic tests isoelectric focusing (IEF) is the most suitable method to separate the proteins of different animal species. Samples studied were taken from raw meat and heat-treated model meat products. The model samples contained meat pulp made of pork, beef, hare and poultry in various proportions. ULTRASCAN XL laser densitometer was used to test the gel base. Two varieties of sample dissolving methods and four kinds of ampholite mixtures were analysed. The results of the experimental series show that the method selected is suitable for the simultaneous detection and qualitative and quantitative determination of the proteins in raw meats of different origin and in products prepared from them.

RECEIPT OF FRESH MILK ACCORDING TO ITS QUALITY AND COMPOSITION

GY. BABELLA and A. UNGER

Hungarian Dairy Research Institute,
H-9200 Mosonmagyaróvár, Lucsony u. 24. Hungary

Improving the hygienic quality parameters of milk and dairy products is a permanent task in every country in the world where buyer's market prevails. This task can be accomplished by improving the quality of fresh milk. In 1984 a new method for determining the quality of milk was introduced in Hungary. In the last 5 years as a joint consequence of the more up-to-date system of requirements and the stimulating prices the quality of fresh milk improved substantially. In these days was the point reached when some of the quality requirements became outworn and lost their stimulating and quality improving effect. Research and development work, taking into account international experiences and expectations, has been aimed at defining the most important parameters characterizing fresh milk, determining the requirement levels, adapting and developing the proper analytical methods. To ensure steady improvement the system of requirements was extended to include somatic cell count, to narrow the limits of total cell count and to increase sanctioning of milk containing inhibitory agents.

Based on the results of these experiments from 1990 a new fresh milk evaluation system is being introduced in several steps in Hungary. The system being valid at present recognises, beyond the hygienic quality, the fat content and quantity in the price paid for milk. This price system accomplished its objective succesfully because it raised the milk economy from the consumer's position into the seller's position. As a result of the stimulating price — while the gross composition improved — the percentage composition moderately diminished. For the future, however, an increase of the products depending on composition (cheese, cottage cheese, powdered milk) may be expected. These factors indicate that the composition of milk may play a more important part in the price paid for it. The research and development work was aimed at marking out the component important as a price factor, the development of price construction most suitable to conditions in Hungary, the adaption of methods suitable for the determination of composition and the development of the price system based on these factors. In accordance with the results of these experiments it is expected that beginning with 1990 beside fat content the protein content will also be a factor taken into account in the price.

METHODS TO ESTABLISH THE QUALITY OF WHEAT AND MILLED WHEAT PRODUCTS

Á. Mosonyi and Gy. Fehér

Research and Development Ltd Co. of the Milling Industry
H-1024 Budapest, Kis Rókus u. 15/b. Hungary

In the wheat and wheat flour processing industry the tendency is to attribute greater importance to satisfying the requirements on quality. To be able to realize optimal utilization of the available wheat according to quality it is necessary to separate wheat batches at receipt according to the purpose of their utilization. Therefore this research program was aimed at the development of methods for the rapid determination of the quality of wheat and its milling products. One question to be studied was the correlation between the expected milling properties, on the one side and the characteristics of the wheat grain according to size and the structure of the grain, on the other. In 1986 in order to accelerate the receipt of wheat according to quality the selection and testing of suitable methods prognosticating the properties of wheat and its milling products, was started. The study of parameters was given preference the determination of which did not involve high cost or permitted the use of instruments already available at the enterprises of the cereal industry.

Out of the methods providing results within the shortest possible time the determination of the SDS sedimentation volume of wheat meal or experimental flour, was found suitable. Taking into account jointly the protein (wet gluten) content and the SDS value of the wheat boundary values of quality were established and thereby the rapid telling of wheat for human consumption from feeding stuff became possible.

During several years taking samples from generally cultivated known wheat varieties the correlation between the year of growth and the quality characteristics, was investigated. In 1989 in several plants the method suggested by the authors was tested and the results were found promising.

DETERMINATION OF THE CARBON ACID AND SACCHAROSE CONTENT OF FRUITS AND VEGETABLES FROM A SINGLE GAS CHROMATOGRAM

J. M. Perl and M. Morvai

Deparment of Inorganic and Analytical Chemistry,
Eötvös Loránd University,
H-1088 Budapest, Múzeum krt. 4/b. Hungary

The determination of quality and quantity of the main components in fruit and vegetable juices is inevitable in following up the changes occurring due to variety, degree of ripeness, conditions of storage.

The starting condition of the comparable and reproducible quality and quantity determination of the main components is the preparation of the matrix. For fruits the preparation of matrix consists in the homogenization of the fruit flesh without the skin. For vegetables preparation consists in the way of extraction of the flesh without skin. The 50–100% differences found in the main components of the same matrix are attributable to differences in the extracts. In the present study the 4:1 volume mixture of water–ethanol or water–methanol was used at room temperature and at the boiling point of the given mixture to extract 2 to 20 g of matrix in 100 cm^3 for 15 min.

The saccharide and carbon acid content of potatoes, carrots and cucumber extracts obtained as described above, was determined as trimethylsilyl-oxime ether or trimethyl-silyl-ester and ether from a single gas chromatogram.

The maximum extractable amounts of fructose, glucose, maltose and possibly raffinose oligosaccharides, C_1–C_{12} fatty acids, C_2–C_{12} aliphatic dicarboxylic acids and numerous hydroxy carboxylic acids, were determined in the matrices.

The conditions and reproducibility of optimal extraction, derivative preparation and gas-chromatographic elution for single matrices were analyzed in detail.

DETERMINATION OF MYCOTOXINS IN THE FORM OF SILYL DERIVATIVES

K. Korány

Department of Food Chemistry and Nutrition, University of Horticulture and Food Industry, H-1118 Budapest, Villányi út 35–43. Hungary

In the frame of the research program the determination of mycotoxins by gas chromatography, in the form of TMS was aimed at. It was attempted to determine the conditions of derivative formation of 4 components: penicillic acid, zearalenon (F-2 toxin), citrinine and ochratoxin A. Of the agents used in actual practice only pure BSTFA and BSTFA: TMCS: benzene were suitable to convert all the four compounds into derivative, unfortunately citrinine and ochratoxin in not quantitatively reproducible form.

As a result of many experiments to extract corn samples a simple repeated extraction with chloroform was found suitable, avoiding losses originating from overfine communition and homogenization and the extraction of too much disturbing matter. In the case of apple juice the additive determination of patulin inner standard was the aim, penicillic acid being used as inner relating substance. Mycotoxins were extracted with diethyl ether from the

samples. After uniting the ether phases they were dried over sodium sulphate, then filtered through folded filter paper and evaporated to dry. The samples mixed with BSTFA–TMCS–benzene (4:1:5) silyling agent formed derivatives and were gas chromatographed.

DETERMINATION OF SIDESTREAM SMOKE YIELD OF CIGARETTES

J. Hamza, S. Makleit and S. Nemes

Tobacco Research Institute,
H-4002 Debrecen, Attila tér 3. Hungary

The correlation between smoking and health came to the fore since the beginning of the 60s. As an effect of strong anti-smoking propaganda effective methods were developed to reduce the amount of main stream smoke of cigarettes.

In recent years the study of the smoking—health relation has been extended over passive smoking, too. Passive smokers inhale the smoke in the ambient air which originates mostly from sidestream smoke (smoke formed between two sniffs). In the first step to reduce sidestream smoke formation the development of reproducible methods of appropriate accuracy, is necessary. Requirements upon the methods are:

— they should not influence the burning of the cigarette;

— they should render possible the complete collection of by-smoke (particles as well as the gas phase);

— simultanously they should render possible the determination of the main smoke according to the specifications of the international standard.

To determine sidestream smoke the method of Proctor and co-workers (*Analyst*, 1988, *113*, 1509–1513) was adapted—with some modifications—which was accepted also by CORESTA as a basis to the international standard. The essential part of the method is a specially formed chimney which at a given exhausting power permits the collection of the sidestream smoke.

The particle fraction is collected on a glass fibre filter and the gas phase in a plastic bag.

The adapted method, judged by the measurements carried out, does not influence the standard smoking conditions.

The accuracy of the method is in agreement with the methods of similar type used in international practice. In the case of alkaloids and CO it is even higher.

Using the method the tar, alkaloid and CO content of the sidestream smoke of the Hungarian high-consumption cigarettes were determined. The data obtained were in good agreement with those found in pertinent literature for cigarettes of similar type.

NEW SOURCES OF PROTEIN AND THEIR UTILIZATION IN THE FOOD INDUSTRY

R. LÁSZTITY and A. SALGÓ

Department of Biochemistry and Food Technology, Technical University, H-111 Budapest, Műegyetem rkp. 3. Hungary

To be able to provide every strata of society with food rich in protein at an acceptable price and to widen the selection of protein based foods renders necessary the industrial production of these foodstuffs. The main trends of research in this field are the following:

— extraction and purification of the new proteins;

— determination of the amino acid composition and biological value of the protein concentrates and isolates thus obtained;

— study of the technofunctional properties of these proteins;

— utilization of these preparations in the meat and other products.

The non-conventional protein sources studied were: germs (wheat, corn, rice), gluten proteins, lupine, millet, sunflower seed, squash seed, tomato seed, mango seed as well as yeast proteins. The optimum parameters of isolating protein from all these sources were determined. A method was developed and patented to remove bitter subtances from lupine protein preparations. Determination of the amino acid composition of the proteins permitted the enlargement of the data-bank, the complex evaluation of these proteins and the optimization of protein compositions of new food products. On the basis of the technofunctional properties successful experiments were carried out for their application in meat, bakery and extruded products.

NUTRITION BIOLOGICAL RESEARCH AIMED AT THE DEVELOPMENT OF SPECIAL DAIRY PRODUCTS

B. SCHAFFER[a], G. PALLAI[a], GY. MÓZSIK[a], S. SZAKÁLY[b], Á. KOVÁCS[b] and G. OBERT[b]

[a] *First Department of Medicine, I. Medical University, H-7624 Pécs, Ifjúság útja 23. Hungary*
[b] *Hungarian Dairy Research Institute, H-7614 Pécs, Tüzér u. 15. Hungary*

Results of the research work carried out jointly at the First Faculty of Internal Medicine, Medical University, Pécs and the Hungarian Dairy Research Institute, Pécs, started about 15 years ago, with the aim of laying down the nutrition biological fundaments of diversification in the milk industry, are summed up as follows:

1. In the course of investigating the problem of fat resorption it was found that this process is affected by the colloidchemical structure. The intensity of resorption is higher related to the continuous phase fat from fat-in water emulsion systems and resorption increases with increasing degree of dispersity of the fat globuls. Clinical control tests have shown that from fat emulsions with a general diameter below 0.5 even sick persons suffering from chronic pancreatitis are capable of fat resorption to such an extent that their fat voidance remains below the chronic value. Based on these results was the butter cream family developed.

2. Protein utilization was compared in relation to processed cheeses contra cream cheeses (without melting salt). Protein utilization from cheeses prepared without melting salt was significantly better. A further advantage of these products that the calcium—phosphor proportion remains physiological (it is not modified by the melting salt in a disadvantageous direction) thus it promotes the integration of Ca in the organism. On the basis of these nutrition biological studies a new family of spreadable cheeses was developed which will be on the market during 1990.

3. Based on the comparative study of osmolarities a series of soft drinks was developed with osmotic pressure similar to that of blood. These soft drinks are based on milk serum having a high natural vitamin and mineral content.

4. It was established that alcohol absorption was substantially lower from cream-liqueurs than from liqueurs not containing fat emulsion. In the case of identical alcohol consumption the alcohol level in the blood depends on the fat content. From drinks of 12% fat content the alcohol level of blood is half as much as from drinks not containing fat.

In the last decade product diversification in the dairy industry was determined by results of nutrition biological research.

POSSIBILITIES OF THE DIVERSIFICATION OF EWE-MILK PRODUCTS

J. Fenyvessy

College Faculty of Food Industry,
University of Horticulture and Food Industry,
H-6724 Szeged, Marx tér 4. Hungary

In recent years cross-breeding experiments have been started to increase the milk yield of ewes. At present all the purchased milk is being made into Kaskaval cheese to be sold abroad. There is a demand in Hungary, too, for the products of ewe-milk. In the course of industrial processing of ewe-milk the factors which in comparison to the processing of cow's milk necessitate the use of different technological operations, have to be taken into account. Ewe-milk

contains a higher concentration of the main components than cow's milk, its microbiological quality is lower and the condition of the fat emulsion is less favourable. Ewe-cheeses manufactured at present are expected to remain marketable products, therefore in diversification of ewe-milk products mainly the whey containing valuable components as the by-product of cheese manufacture has to be taken into consideration. Ewe whey is of higher solids content than cow's whey because of its higher protein and fat content.

The possible ways of utilizing the valuable components of whey are the following: Utilization of the milk fat obtained by scimming the whey, separation of the protein content by heat treatment, preparation of whey cheeses, the use of denatured proteins in processed cheese.

In the knowledge of the above described processes essays were made to develop new ewe-milk products. In developing the new technologies the microbiological safety of the products and the long period of quality retention, was taken into account. A cream-like cheese filled into tubes and a kind of processed cheese were developed and are available in the retail trade.

With the aim of diversification fresh cheeses an "orda-type" cheese was prepared from ewe-milk and whey and the critical points of the technological process were investigated. Research work was extended over the concentrate produced by the ultra-filtration of whey and possibilities of its use in creamed white cheese and creamed cottage cheese, etc.

PRODUCTION OF A COMPLEX ADDITIVE FOR THE MEAT INDUSTRY FROM HUNGARIAN RAW MATERIAL

T. Huszka, J. Fenyvessy, E. Fehér-Patkós, M. Fekete-Halász, E. Pallagi-Bánfalvi and I. Jankó

College Faculty of Food Industry, University of Horticulture and Food Industry, H-6724 Szeged, Marx tér 4. Hungary

The manufacture of up-to-date meat products is inconcievable without the use of additives. The reason for this is the increased rate of technological processes or the reduced operation period of the processing machinery.

In the interest of consumers' rights it is also important to be able to stabilize the consistency and colour of meat products. Unfortunately most of the additives are imported although they could be prepared from Hungarian raw materials, too.

The authors set themselves the task of developing a complex additive for the meat and poultry industries composed from Hungarian materials and of the same value as the imported additives. The result of this work was the

OPTIMIT additive composed from several components. The component responsible for consistency, LAKTOPROT-GÉL is prepared by a microbiological process from milk by setting the appropriate pH and applying melting salt to obtain Na-caseinate of gel consistency. Since this product does not get any kind of heat treatment its native protein content retains its technofunctional properties better than other caseinates and its water and fat emulsifying capacity is extremely good.

The colour improving component, COHEM is a lyophylised but heat-stable hemoglobin derivative and permits of reducing the NO_2 requirement in the meat products. The consistency improving additives do not contain myoglobin, therefore they cause a lighter colour and reduction of colour stability of the meat products.

The hemoglobin containing substance retains its red colour even during heat treatment, therefore it serves as a colour stabilizing component. To improve the flavour and aroma of meat products the ZSELAROM additive was prepared by hydrolysis of the connective tissue proteins in chicken feet and the addition of amino acids and other substances. The OPTIMIT complex additive contains all the enumerated agents, however, these can be applied one by one, as well.

UTILIZATION OF SOY BRAN FOR HUMAN CONSUMPTION

E. Schöbel, K. Doborján and J. Bogdán

Scientific and Production Association for Protein Technology, H-1536 Budapest, Pf. 340. Hungary

In the last decades the cultivation in Hungary of soy bean as a source of high value protein became of general interest. The husks as a by-product were used so far as an animal fodder. To use it for human consumption has been started only recently.

In the frame of a national level research program it was possible to create the conditions for the production of food quality soy bran. The authors studied the composition of soy bran and its functional properties. The results of these studies are the following:

— Soy bran being very rich in food fibre may be used to balance the setbacks of fibre deficient nutrition. The fibre content was found to be 58–65% within which the water-insoluble part amounts to 70%.

— The bran is a fine pulver of yellowish-brown colour and its taste reminds of raw soy bean.

— The protein content is similar to that of wheat bran but the fat content is lower.

— The high ash content shows the presence of high amount of minerals. As regards the mineral content the essential micro and macroelements are present in a significant quantity. It is an ample source of iron, chrome, magnesium and potassium. At the same time toxic elements are present in the amount below the permissible level.

— It is free of phitic acid and its trypsin inhibitory activity does not exceed the permitted value. Because of its low raffinose, stachiose content it practically has no flatulent effect.

— The water binding capacity of soy bran is higher than that of wheat bran.

— Of its functional properties a high water binding capacity and emulsifying activity as well as a medium oil binding capacity, are significant. In the soy bran–fat–water emulsions of 1:5:5 and 1:10:10 proportion the stability of the emulsion as established by cooking test, is complete.

— Product diversification experiments were carried out in the dairy and baking industry to produce bran breads and yoghurt enriched with apple and soy bran. It is intented to continue diversification by extending the field of application.

BAKERY PRODUCTS OF HIGHER NUTRITION BIOLOGICAL VALUE

J. Moór

Joint Enterprise for the Baking Industry,
H-1117 Budapest, Dombovári út 5–7. Hungary

The nutrition biologically higher value bakery products developed were the following:

1. Products of increased fibre and vitamin content:

Coarse grained rye loaf of 0.50 kg;

Coarse wheat meal loaf with sesame seeds of 0.60 kg;

Loaf baked from coarse wheat and rye meal with caraway seeds of 0.50 kg

Roll, cut in

Crescent shaped roll

White bread enriched with fibre of 0.60 kg

All these products are prepared from fully milled wheat or/and rye flour or durum wheat bran. Their fibre content is 2.5–4.5 times higher than in ordinary white bread and the vitamin content is significant, too. Wrapped products have longer shelf life.

2. Products of higher protein content and biological value:

Natural soy bread of 0.70 kg

Natural soy and cheese bar

The soy content of the products is 25 or 10%. This increases their protein content by 30–40 rel.% and their biological value is higher, too.

3. Products of increased vitamin B content:

Sponge finger from aleurone flour with honey

Sponge finger from aleurone flour with fat

The products are chemically and physically made looser. Their thiamine content is 7.5 fold, riboflavin content 1.5 fold, niacin content 3 fold and pyridoxine content 2.5–6.5 fold higher than in bakery products made of ordinary bread flour. The products are recommended for children.

NUTRITION BIOLOGICAL IMPORTANCE AND ROLE OF "GARDEN" FIBRE-ENRICHED FRUIT JUICES

J. Vajay

Badacsonyvidéki Pincegazdaság,
H-8230 Balatonfüred, Zrínyi u. 11. Hungary

The "Garden" products are made in Szombathely and distributed from there.

Retail packaging is carried out on a PKL aseptic system. The juice is heat treated in a counter current, lamellar pasteurizer. The packaging material is sterilized with hydrogen peroxide and heat treatment. Cleaning is done with the automated CIP system. The fruit juices rich in fibre can be considered as purposeful products because beside their high sensory value their carbohydrate and energy content is low. The mixture of aspartam and K-acesulfan is used for sweetening. Neither preservative nor colouring substance are used.

It can be safely consumed in slimming cure or by diabetics, it does not produce "depot fat" and does contain added carbohydrate. It has a noteworthy mineral and vitamin content and thereby helps in the re-establishing of the balance of nutrient proportions. The 40% fruit content and the favourable fibre content in addition to the high compositional value is wholesome, prevents deseases caused by civilisation and creates harmonious general condition.

The profilactic effect of the fibre fraction and its role as carrier and retainer is of high importance.

The products are of a standard quality in spite of the yearly fluctuations of the raw material.

Great important is attached to keeping their products uniform, biologically clean and guaranteed delivery on time.

MAGNESIUM AND TRACE ELEMENT ENRICHED FRUIT DRINKS AND JAMS

Z. SÁNDOR, M. TÖLGYES and B. LAKATOS

Central Research Institute for Chemistry of the Hungarian Academy of Sciences, H-1025 Budapest, Pusztaszeri út 59–67. Hungary

It is well known that the micro- and macro-element content and thus the metal content in our food in general does not cover our daily requirements. The lack of metals (Mg, Fe, Zn, Mn, Cu, Co, Mo, Cr, Ni, etc.) indispensable for the living organisms reduces the activity of proteins, enzymes, hormons and other biologically active agents which are needed for the functioning of metabolism and the control of other processes. The lack of certain metals may be one of the reasons of a number of illnesses. On the other hand the excessive consumption of some other elements may be responsible for other deseases. Ca is for instance one of these elements. Its antagonist, Mg, is frequently below the necessary level in our daily food (about 420 mg per 70 kg body mass). Magnesium activates 325 enzymes and is needed in every energy producing process in the human body. The most well known deficiency disease is anemia due to the lack of sufficient iron. The deficiency of all the above mentioned metals indicates the production of enriched foods.

In the second half of the 1970s a process for the production of metal polygalacturonates was developed at the Central Research Institute for Chemistry of the Hungarian Academy of Sciences and patented. In 1988 further processes were patented to produce and utilize basic polygalacturonate. This new type additive has a higher specific metal content and its production technology is substantionally simpler and cheaper than the earlier process. The starting material of the additive is pectin obtained from fruits an other plants. The reactive OH groups of the basic metal-polygalacturonates react (during the production of fibre containing fruit drinks and jams) with the indigenous acids of the fruit, including the free carboxyl groups of the pectin content as well as added acids. Instead of citric acid malic or tartaric acid is used as acidifying agent because citric acid is disposed towards forming metal complexes of high stability and thus decreasing their biological utility. As a result of these acid-base reactions the products made of fruit contain metals in a natural form which, therefore, are easily utilized. The first products marketed are: magnesium-enriched jam (100 mg added Mg per 100 g jam), fruit juice rich in fibre (100 mg added Mg per 0.5 litre juice). Fruit drinks containing several metals are expected to be put on the market soon.

NATURAL APPLE AROMA CONCENTRATE

M. Petró-Turza, I. Szárföldi-Szalma, K. Füzesi-Kardos,
E. Madarassy-Mersich and Gy. Teleky-Vámossy

Central Food Research Institute,
H-1012 Budapest, Herman Ottó út 15. Hungary

The watery aromas produced by condensation of the steam formed during the evaporation of fruit juices can be marketed mostly only on the home market. These condensates are characterized by large storage room requirement, low keeping quality, a very limited utility and accordingly a relatively low price. A product of this type is manufactured in Hungary only from apple. The manufacturers of this kind of product or those possessing the conditions of manufacture are fully aware of these setbacks. Therefore in the majority of plants the aroma recovering units attached to the fruit juice evaporating line are not used at all or only during a short period during the fruit processing season. In consequence of this the valuable natural fruit aromas, more and more in demand all over the world get in the sewer or in the air and are not utilized.

To the reversion of this situation a new patent of the Central Food Research Institute offers possibility by providing a new technique to the concentration of these watery aroma solutions.

The quality parameters of the product made by the patented process on the new producing equipment, are given in detail. Using apple juice as an example the difference between the sensory and compositional parameters of the original watery aroma and the concentrate, are described. The efficiency and advantages of the new process in comparison with other methods used earlier to concentrate aromas, are demonstrated.

NATURAL FOODS FROM JERUSALEM ARTICHOKE TUBERS

J. Barta, B. Gion and Sz. Török

Department of Canning Technology,
University of Horticulture and Food Industry,
H-1118 Budapest, Ménesi út 45. Hungary

The edible carbohydrate of Jerusalem artichokes consists mainly of inulin which can be hydrolized to fructose. The inulin content of the tubers harvested at the optimal time consists of fructose to 75–85%. Fructose is 20% sweeter than saccharose, thus, upon consumption it takes less energy into the body.

The family of products produced by processing Jerusalem artichokes is the following:

— Not purified Jerusalem artichoke concentrate of the enzymatically hydrolyzed tubers (75–85% fructose of the dry subtances);

— Jerusalem artichoke concentrate, similar to honey, purified by ion exchange (75–85% fructose in dry matter);

— Purified fructose syrup (96–98% fructose in dry subtance);

— Crystallized fructose;

— Jerusalem artichoke pulver rich in inulin and fibre;

— Dried, pressed Jerusalem artichoke granulate;

— Dried Jerusalem artichoke cubes;

— Deep-frozen Jerusalem artichoke cubes;

— Heat-preserved hydrolyzed Jerusalem artichoke pulp.

Of the different technological processes the enzymic hydrolysis is discussed, which permits the manufacture of the concentrate rich in fructose, containing all the components of Jerusalem artichokes (micro- and macro-elements alike).

The enzymic hydrolysis was carried out with two enzyme preparations of different origin. The total sugar content related to the dry subtance of the hydrolysate obtained with ROHALASE I-10X enzyme (of kluy-veromyces yeast origin) was 79%. In the hydrolysate obtained with Novo Inulinase of mould origin (Aspergillus) was 86.8%. The total sugar content of the syrup purified by ion exchange and obtained by acid hydrolysis was 98.8%.

The product by enzymic hydrolysis was 5–10 times darker than the product purified by ion exchange. Darkening can be eliminated to a certain extent by inactivating the polyphenol oxidases.

In the enzyme-hydrolyzed juices and in their concentrates the macro- and micro-elements are retained while in the course of purification by ion exchange the mineral content gets substantially reduced. The taste of the concentrate of enzymic-hydrolyzed juice differs from the usual plain sweet taste, it has the characteristic taste of the Jerusalem artichoke tubers, which can be explained by the presence of amino acids and minerals.

The by-product of juice production, the pressed Jerusalem artichoke marc (crushed artichokes) is dried and forms a powder of relatively high fibre content and can be used in the dietetic products of the baking industry.

By drying the tubers a product of high biological value is obtained which can also be utilized in diabetic products. The diced, appropriately prepared tubers dried in hot air give a product of high inulin content. It can be used as an additive to salads and other vegetable products.

APPLICATION OF A FINITE ELEMENT CALCULATION METHOD IN STERILIZING CONDUCTION HEATED CANNED PRODUCTS

I. KÖRMENDY, GY. PÁTKAI, M. ERDÉLYI and L. MÉSZÁROS

Department of Canning Technology,
University of Horticulture and Food Industry,
H-1118 Budapest, Ménesi út 45. Hungary

A finite element method, explicit type and stepping forward in time has been used to calculate the temperature field in cylindrical cans when outer temperature and heat transfer coefficient were changing with time. The use of the method is justified if heat propagates within the can dominatingly by conduction.

The variation of the quality attributes (number of surviving microorganism, reduction of vitamin content) in consequence of the variation of the temperature field and depending on the heat treatment time can also be calculated by this method. The method was complemented by a procedure minimizing the mean quadratic difference of measured and calculated temperatures. Thereby it became possible to determine and compare with data in the literature the more important thermophysical constants (outside heat transfer coefficient, heat conductivity and diffusivity in the food) from laboratory and industrial experiments. Baby foods, canned meat products, tomato puree, cooked egg-yolk, solid copper cylinder have been investigated.

As for the industrial equipments, hydrostatic sterilizers with divided columns and a horizontal autoclave were investigated. Comparing the finite element method with a method based on the analytical solution the results showed an excellent correspondance. However, the analytical method is unsuitable for the solution of most of the industrial tasks.

EXPANSIVE VACUUM COOLING AND VACUUMIZING IN THE FOOD INDUSTRY

A. TALLIÁN, A. VERBA, M. SZABÓ and B. KATONA

Chemical and Food Engineering Department,
Technical University,
H-1111 Budapest, Műegyetem rkp. 3. Hungary

The expansive vacuum cooling process can be applied in food industrial or chemical technologies. The rapid cooling of cooling water, various liquid products, watery solutions can be resolved economically by this technique. For instance in fruit juice manufacture rapid cooling of the juice concentrate

improves its quality. Alcohol distilling equipment can be intensified in the summer with cooling water thus cooled to 5–20 °C.

Expansive vacuum cooling requires the exhaustion of a high amount of vapor. This can be economically done by the use of a steam-jet vacuum pump. The pump has no mobile fixture and is easy to manufacture. The design of such fixture requires special theoretical knowledge and practice in this field. Members of the Department developed a procedure for the dimensioning of steam jet pumps. Laboratory results and even industrial applications prove the reliability of the procedure. From the aspect of operation the simplest equipment is a single-step steam jet pump expansive vacuum cooler operated by a barometric condenser. The barometric condenser is de-aerated economically with a water-ringed vacuum pump connected to a single-step steam jet pump vacuum equipment.

The authors prepared the computerized model of the above described equipment. The model renders help to the caloric operational dimensioning of the expansive vacuum cooler.

APPLICATION OF SUPERCRITICAL EXTRACTION IN THE FOOD INDUSTRY

B. Simándi, J. Sawinsky, A. Deák and S. Kemény

Department of Chemical Engineering, Technical University, H-1111 Budapest, Műegyetem rkp. 3. Hungary

Supercritical extraction replaces the traditional separating processes in steadily growing fields. The advantage of the process is that the product is purer and of better quality. It renders possible the extraction of a number of natural components otherwise lost. The solvent most frequently used for extraction is carbon dioxide because it is not detrimental to human health, cheap, not inflammable and does not contaminate the environment. Of the innumerable possibilities of its use some examples are shown to present the advantages and possibilities. The authors studied this technique from the calculation of phase equilibrium to the final operation. The first step was to prepare a program for calculating the supercritical solubility of solid materials by the application of cubic equations of state. Starting from measurement data obtained in the literature the interaction coefficients of the most important cubic equations of state were determined by parameter estimating program and the equations of state studied were evaluated on the basis of square sums of difference.

Laboratory experiments were carried out to extract the essential oil content of orange peel. The essential oil was extracted from freshly peeled and

cut up or dried at room temperature and then cut up orange peel by carbon dioxide. The effects of the parameters of extraction and separation on the quantity and composition of the extract (temperature, pressure) were determined. The mass flow of the solvent and the time of extraction were also varied. The extract was compared to the cold pressed oil available on the market.

RESULTS OF MEMBRANE FILTER DEVELOPMENT

L. Mester and E. Godek

Central Food Research Institute,
H-1022 Budapest, Herman Ottó út 15. Hungary

Research and development work has been carried out at the Central Food Research Institute on filter membranes, membrane filter module and equipment and membrane separation technologies with a view to their application in the food industry.

The first task to resolve was the problem of preparing plane membrane filters. On the basis of their experimental results the manufacture of membranes was realized at the Institute. The plane membranes were of 40, 50 and 100 cm width and any length (max. 300 m) of maximum 100 m^2 per h capacity. The basic material of the membranes was cellulose acetate and polysulfone. The cellulose acetate membranes cover in the pH range 3–8, in the temperature range of 0 to 30 °C the reverse osmosis domain and from 95% NaCl retention to 20 thousand dalton cutting value the ultrafilter range. From cellulose acetate membrane 8 marketable types were developed with guaranteed parameters. The polysulfone membranes fall in the 5 thousand to 50 thousand dalton cutting value ultra-filter region and do not deteriorate in the 1–13 pH range and the 0–75 °C temperature range. Manufacture was typified also to the more important separation limits. The protein retaining polysulfone ultrafilter is used in the widest range of the food industry. These provide above 99.9% milk protein retention at 35–40 l per m^2 h output (6 bar, 55 °C) while the aqueous fluxus at similar parameters 600–700 l per m^2 h.

In 1989, as the result of their own experiments the manufacture of ultrafilter spiral modules of 6 m^2 useful surface, was also started. These are marketed in compliance with the guaranteed conditions (selectivity, output, life expectancy, etc.) of the foreign firms manufacturing spiral module inserts.

INSTANT FRUIT POWDERS AND CHOPPED UP PIECES PRODUCED BY MICROWAVE VACUUM DRYING

J. DÖRNYEI

COMPACK Commercial Packaging Co.,
H-1078 Budapest, Landler J. u. 23–25. Hungary

Vacuum drying produces thermoplastic foods rich in aroma substances. When the drying temperature is produced by microwaves in the vacuum drier the heating rate increases 20–30 times since microwaves heat the porous foamy mass evenly. In the case of moisture removal of such intensity it is extremely difficult to retain the flavor and aroma substances in the dried product. Various materials were dried by a series of experiments in microwave vacuum drier.

In the course of this research work it was established that taste and flavour can be retained during microwave vacuum drying, if:

— the basic concentrate has minimally 65% solids content;

— on the surface of the foam layer formed and drying a semipermeable biological membrane evolves;

— the membrane can be developed on the surface of the foam layer from different components added to the concentrate previous to drying;

— after mixing in the additives aroma concentrate or moisture-free aroma can also be added;

— the concentrate mixed with the additives is to be within predetermined physical-chemical parameter limits;

— microwave heating is produced by generating, perpendicular to the foam layer continuous electromagnetic (cW) radiation of even field intensity.

The minimum permissible vacuum during microwave vacuum drying should be 10 mbar (absolute pressure), the maximum product temperature during drying 35 °C at which values the specific water evaporation rate of 4–5 kg water per m^2 per h can be ensured.

The above enumerated positive characters hold good only partially with larger pieces of food, e.g. in final drying of apple cubes.

INCREASING THE STORAGE LIFE OF MINCED MEAT PRODUCTS BY THE COMBINATION OF ANTIMICROBIAL EFFECTS

J. FARKAS, D. BÁNÁTI, É. ANDRÁSSY, S. BARABÁSSY and K. HORTI

Institute for Preservation and Livestock Products Technology, University of Horticulture and Food Industry, H-1118 Budapest, Ménesi út 45. Hungary

It was investigated how far the reduction of pH or water activity by various additives in themselves or in combination with pasteurizing radiation can extend the refrigerated storage life of vacuum packaged minced pork or tenderloin rolls.

The reduction of pH with ascorbic acid or acid pyrophosphate (pH 6→5.4) caused the storage life of minced pork at +2 °C to be extended from the original two weeks to 19 days. Combined with radiation treatment of 1 kGy it was extended to three weeks. Further extension of the storage life by further reducing pH was not possible because of quality deterioration of non-microbiological origin. The storage life of vacuum packaged tenderloin rolls at +2 °C was originally 12 days and this was extended to 1 month by the addition of 5% glycine slightly reducing thereby the water activity or by an irradiation dose of 2 kGy. If the pH of the vacuum packaged product was reduced to 5.4 by the addition of lactic acid and in addition 5% glycine or treatment with 2 kGy radiation the storage life was extended to 3 months. The storage life of tenderloin rolls at 4 °C was only about 5 days which could be extended two weeks by reducing the pH to 5.4 with lactic acid, while radiation treatment with 2 kGy extended it to 3 weeks. The combination of these two treatment extended the storage life to 4 weeks.

MODERN SUGAR DRYING AND COOLING EQUIPMENT

J. BAKONYI

AGROBER Consulting Engineering Office for Agriculture and Food Industry, H-1111 Budapest, Budafoki út 59. Hungary

In the frame of the research study conditions of sugar drying and the requirements of storage in silo in relation to the initial relative humidity, was investigated. The moisture content of the sugar to be stored must not exceed 0.03% and the temperature 30–35 °C. Different methods of drying: in drying cylinder with hot air, and the drying cylinder fitted with series of buckets, a joint development of the machine factory and the sugar factory, were compared.

The 400 tons per day and 720 tons per day capacity variants of the drying equipment are successfully used in a number of sugar factories in Hungary and abroad. The intensity and net efficiency of drying is satisfactory. It is worth noting that when the first drying machine was installed over-drying was considered the danger. It was proven in the practice that the sugar dried with this machine met all the storage requirements in a silo.

PRODUCTION OF ADDITIVE BASED ON STARCH

L. Ludvig, F. Ledniczky and Gy. Madarász

Research Institute of the Alcohol Industry, H-1089 Budapest, Diószeghy S. u. 8. Hungary

Food additives of various composition are used by the different branches of the food industry. The manufacture of modern food products often requires the use of consistency modifying additives. This requirements is at present covered by imported products. A definite important component of the consistency modifying agents is starch appropriately modified or some thickening agent of plant or animal origin. By composing the additive from the desirable substances the consistency of the product can achieve the necessary viscosity, a steady viscosity or whatever change is desired. The requirement on the additive may be its reaction to acids, heat, mechanical effect, etc. In recent times at the Research Institute the manufacturing technology of different starch derivatives (oxidized, phosphate, acetate and water soluble starches, etc.) have been developed. Some of these products were suitable for use in mixtures. The possibility of their use in the milk, confectionery, refrigerating, baking and canning industries has been studied.

Some of these products are already manufactured by the industry and successfully replace substances hitherto imported.

STABILIZATION OF CATSUPS WITHOUT PRESERVATIVE

J. Szigeti[a] and O. Reichart[b]

[a] *Pannon Agricultural University, Department of Food Technology and Microbiology, H-9200 Mosonmagyaróvár, Lucsony u. 15–17. Hungary*
[b] *University of Horticulture and Food Industry, H-1118 Budapest, Ménesi út 45. Hungary*

The research work was aimed at realizing various catsups manufacturing without the use of preservatives.

The main results of the experiments were the following:

— It was established that the white spots in catsups are caused by an atypical Lactobacillus brevis strain. On the other hand moulding in tomato puree and catsup is generally caused by Penicillium glaucum strains.

— The non-dissociated organic acid proportion, pH and water activity combination at which the spoilage causing microorganisms in a given product are destroyed, was determined.

— The significance of differences between total viable cell count, Lactobacillus count and mould count in the samples treated in different ways was calculated by analysis of variance.

— The integrated buffer capacity of the different products was determined in relation to organic and inorganic acids.

— The microbiological stabilisation of tomato puree in barrels and different catsups in jars was proven in industrial storage tests.

The results of the experiments was that the authors succeeded in coordinating the pH, organic acid and water activity values of tomato puree and of the catsups made of it so that the microorganisms infecting and causing the spoilage of these products, were destroyed. Thus it became superfluous to apply heat for killing the microorganisms and it was necessary only to activate the factors responsible for consistency.

REDUCING THE MALIC ACID CONTENT IN WINES BY A COMBINED BIOLOGICAL PROCESS

I. Magyar and I. Panyik

Department of Oenology, University of Horticulture and Food Industry, H-1118 Budapest, Villányi út 35–43. Hungary

The harmony of acids in red wines and their microbiological stability is advantageously affected by breaking down their malic acid content. A widely applied method to achieve this result is the malolactic fermentation of the wine carried out by lactic acid bacteria. However, this process is difficult to control.

An experiment was carried out to use an alternative biotechnological process using the yeast strain Schizosaccharomyces pombe immobilized in Ca-alginate.

In batch and continuous fermentation experiments, in red wines free of sugar and of various sugar contents it was found that by the use of the fixed cell ciocatalyst a partial (2.5–3 g per l) malic acid reduction can be achieved without harming the quality of the wine. The specific activity of the biocatalyst, that is the amount of malic acid decomposed in 1 h by 1 g catalyst,

depending on the experimental conditions, was 0.2–1.7 mg. The mechanical and operational stability of the gel proved satisfactory. In the second half of the experiment this process was combined with the lactic acid bacterial, malolactic fermentation. The prefermentation with fixed yeast provided favourable conditions for the proliferation of lactic acid bacteria and accelerated substantially the starting up of the malolactic fermentation. The sensory quality of wines freed by the combined process (yeasts-bacteria) from malic acid was equal to or better than that of the wines fermented only by bacteria. To the industrial utilization of the process it is necessary to increase the specific capacity of the biocatalyst and the elaboration of the conditions of large scale production, pretreatment and storage.

COMPUTER CONTROLLED PILOT SCALE GROUND PAPRIKA PRODUCTION LINE

T. Huszka, A. Véha, J. Gyeviki, Zs. Hovorka and M. Halász-Fekete

College Faculty of Food Industry, University of Horticulture and Food Industry, H-6724 Szeged, Marx tér 4. Hungary

In the technology of ground paprika production computer control was first introduced in the experimental plant of the College Faculty for Food Industry in 1985. To characterize the system then developed it can be said that the control of partial processes were individually resolved with ZX Spectrum, C-64, PDV-38 type computers of low capacity.

Within the frame of research program G-8 it became possible by the use of IBM compatible computer and the Turbo C program language to build up a uniform system meeting the control requirements of the future industrial production processes.

The present system includes the storage data basis treating and the formula optimizing system, as well as the control functions of weighing, mixing and grinding processes.

The system built up at present consists of the following hardware units ACER 910 type IBM AT computer, 1 MB RAM, 40 MB Winchester, EGA polychrome and HERCULES monochrome graphic monitor. The strict adherence to the quality specifications in the manufacturing line was increased by the development of an adequately safe statistical sampling program related to the composition characteristics. The formulation system according to colour is the first one in the world. Computer control in the manufacture of ground paprika is an advantage for the Hungarian industry over the foreign competitors because it improves the efficacy of production and the uniform quality of the product.

FOOD QUALITY SAVING PACKAGING-TECHNICAL METHODS

I. VARSÁNYI

Central Food Research Institute,
H-1022 Budapest, Herman Ottó u. 15. Hungary

Changes of the raw materials, semi- and final products of the food industry as a function of storage time are usually of negative character. Thus, their sensory and/or biological value decreases and some of their properties become modified.

The technological processes applied to the products to save their quality are efficient if they form a protective wrapping taking into account the possible quality changes and the factors responsible for them.

The analysis of the causes of quality changes in foods in connection with packaging has shown that they can be traced back to chemical, physical or microbiological processes. Also the influence of environmental temperature must not be disregarded.

Upon analysis of the correlation between the changes in food and the wrapping applied it was found that the relationship was stochastic. The kinetics and mechanism of spoilage, including those of microbiological origin depends largely and directly on the gas and vapour permeability of the packaging material, indirectly on the permeability of light and the presence of heavy metals. Sufficiently impermeable packaging materials permit the use of vacuum or protective gas. By means of these methods the reduction of spoilage rate can be achieved.

INTERACTION BETWEEN PLASTIC PACKAGING MATERIALS AND THE FOOD IN CONTACT WITH THEM

M. BÖRÖCZ-SZABÓ and F. BOROSS

Central Food Research Institute,
H-1024 Budapest, Herman Ottó u. 15. Hungary

Plastic materials play an important role in food packaging as independent material or laminated with other materials. The toxic effect of plastics in direct contact with foods or the non-toxic effect on the sensory properties of foods has been the subject of many investigations.

Less is known, however, of compounds diffusing into the plastic packaging materials or the innermost layer of laminated sheets. The authors investigated the volatile compounds of apple juice diffusing the innermost layer of laminates used as packaging materials. It was found that the aroma sub-

stances of apples most characteristic, the esther derivatives infiltrate the plastic layer and are bond by it. Subsequent to appropriate preparation they can be detected by gas chromatography.

This phenomenon is of importance in the case of foods of lower aroma substance reserves, because the loss of some of their aroma content may lead to a quality change or a change in the taste and smell profile.

TEXTURE AND TECHNOFUNCTIONAL PROPERTIES OF SYNTHETIC CASINGS

T. Huszka, K. Bajusz-Kabók, L. Fehér and P. Záhonyi-Racs

College Faculty of Food Industry,
University of Horticulture and Food Industry,
H-6724 Szeged, Marx tér 4. Hungary

The Hungarian meat industry utilizes a steadily increasing quantity of synthetic casings and these come from import. Their texture, however, is not really known, although this is responsible for their functional properties being of great importance in their technical application.

About the wetting and adhesion energy between the synthetic casings and the raw meat mixture filled into them there is very little published in the literature. Thus, the rim angle of the water–fat–protein emulsion to different types of synthetic casings was experimentally studied with an ERMA type instrument. The data measured were used to calculate on the basis of the Young equation, the adhesion energy of the raw mixture. On pictures of synthetic casings taken with light microscope or scanning electromicroscope textural differences were observed which were in agreement with energy measurements. In heat treated meat products the denatured proteins caused a new state in relation to adhesion. In the course of these experiments the energy needed to remove the coagulated layer from the casing, was measured. Data obtained in the course of this study may be made use of in the manufacture of synthetic casings in Hungary or in the evaluation of imported casings.

DEVELOPMENT IN STORAGE TECHNOLOGY

E. Ringbauer

AGROBER Consulting Engineering Office for Agriculture and Food Industry,
H-1111 Budapest, Budafoki út 59. Hungary

A storehouse is the place where the goods have to be kept and be handled during the necessary period at the lowest possible expense. The technical innovations introduced in the last few years in food stores or being under realiza-

tion, are described. The two important requirements on storage technology are: reduction of storage expenses and at the same time rapid serving of customers. In both cases it is necessary to optimize storage technology, organization of the storage and information flow. The organization has to render possible the flow of goods without the accompanying documentation. The paper discusses the following subjects:

Delivery system at the RINGA meat processing enterprise,
New application possibilities of space saving portable racking,
Palletized storage based on FI-FO principle,
Elevated storage in the confectionary and refrigeration industries,
Automated overhead storage in the meat industry.

APPLICATION OF SWEETENING AGENTS AND SUGAR SUBSTITUTES IN THE FOOD INDUSTRY WITH SPECIAL REFERENCE TO ECONOMIC PROBLEMS

E. Szabó and M. Kispál

Central Food Research Institute,
H-1022 Budapest, Herman Ottó u. 15. Hungary

The steady increase of the number of those suffering from diabetes and obesity raises the problem of widening in every country the assortment and volume of diabetic foods and/or foods of reduced energy content.

This process is perceptible in Hungary, too. Its feasibility depends on the coordination of a high number of viewpoints (health, technological, economical, etc.) and on extensive knowledge.

The most important aspects of decision making are outlined but only the questions related to economy are discussed in detail. The development of expenses in various products in relation to the traditional composition and the synergic effect of the combination of different sweetening agents on the economy of the product, were investigated. The analyses were based on the research work carried out in this field at the Central Food Research Institute.

It was established that beside the two sweetening agents, saccharin and cyclamate, cheaper than sugar, two newer sugar substitutes, less known in Hungary, aspartam and acesulfam-K in appropriate combination can be applied in diabetic and low energy foods at competitive prices. In the knowledge about these sweetening agents the Research Institute may be of help to the industry in developing the desired combinations. The specific character of these products requires a complex treatment of the problems and also a more intensive health propaganda on the part of the Government.

INTERESTS INVOLVED IN THE VERTICUM OF THE GOOSE BRANCH

L. DINYA and A. SZABÓ-TÜRKÖSSY

College Faculty of Food Industry,
University of Horticulture and Food Industry,
H-6724 Szeged, Marx tér 7. Hungary

Competitiveness of the products of the Hungarian goose processing exceeds any other product of agriculture on foreign hard currency markets. The authors investigated the reason for this favourable position and the strategical steps necessary to improve the harmony of interests throughout the verticum of this branch. On studying the structure of production and marketing it was found that interests clash at every step. At the individual steps of the goose processing branch, from stock-breeding, through feeding stuff production to retail marketing the connections are interwoven by conflicting interests. Closer structural integration is not characteristic of this branch. Connections are mostly based on bilateral contracts (seldom on multilateral ones) and processing is carried out in individual systems of interest. At each step of production specific problems arise which form a sphere of interest around it. There are problems with the character of components of feedstuffs and the deseases caused in geese by unsuitable feed. Stock breeding lacks a long distant reasoned breeding policy. The efficiency of large scale stock breeding is declining and so does the efficiency of goods production. Due to different reasons the production technology fails to produce goods of desirable competitiveness. Export is transacted practically through a single channel.

Because of the situation described above it would be expedient to elucidate the added value arising at every step of the verticum (also the income) and by the realization of new forms of integration connecting the separate interests to the marketability of the final product.

FOOD MARKETING TASKS ON THE HOME MARKET

J. VIRÁG, G. VÁRHELYI, A. SZABÓ-TÜRKÖSSY and I. RÓZSAHEGYI

College Faculty of Food Industry,
University of Horticulture and Food Industry,
H-6724 Szeged, Marx tér 4. Hungary

Marketing is a deliberately undertaken business aspiration and managerial behaviour. It serves to promote the marketability of the enterprise and by means of organizing the market enables meeting more fully the demands and a more efficient economy.

Marketability does not pertain to goods only, it relates to the final product, the enterprise, the economic unit, thus it is the result of the operation of the whole business system.

Food marketing activity has a number of specific characters which can be traced back to particularities of agricultural production, processing and consumption.

Most of agricultural produces are of large volume, therefore their transport and storage are expensive between location of cultivation and marketing, or processing and the consumer.

A part of agricultural produce is seasonal, their consumption is however, continuous. The problem arising during transport and storage is that most foods are perishable. The production of a great number of plants of similar profile is confronted with a demand frittered away. These specific characteristics distinguish the study of food marketing from the marketing of other industrial products.

More than half of the products of Hungarian food industry are sold on the home market. The wholesale trade has the disposal of little more than one third of the goods and nearly two-thirds are sold by the retail trade. The analysis of the food marketing sub-system has shown the inadequacy of practical food marketing and in certain cases its non-existence. The production and selling functions are separated since the majority of food products are sold through commercial channels.

(Marketing directly to the population does not reach 3%.)

BOOK REVIEWS

Packaging

Technik, Praxilösungen, Perspektiven des Verpackens aus Industrie und Wissenschaft

1. Ausgabe

D. BERNDT (Ed.)

Vulkan Verlag, Essen, 1990, 382 pages

The handbook gives a good survey on the packaging materials, various packaging technologies, techniques and on packaging machines, systems and lines. The book contains 6 chapters, an introduction, a list of advertisers and a subject index.

The first chapter deals with various normatives, regulations, laws and their trends of the 90's. The second chapter is about packaging materials, containers and about auxiliary packaging materials, mainly in respect of paper, metal and glass. Chapter 3 discusses the various packaging techniques, including the production of various containers. Special emphasis is given to corrosion protection, to coffee packaging technique, to welding and sealing of packaging materials made of aluminium, to the packaging of baking products and also to the aseptic packaging of foods. The fourth chapter deals in details with packing machines, equipments, lines and systems also with automatization, application of electronics in packaging technique, computerized measuring, signalling and control systems, vacuum packaging, pallet formation by robot technique and with aseptic packaging technique. The fifth chapter is very timely, it analyses the connections between packaging and environment with special regard to the more and more widely applied plastic and plastic combined packages. The last, sixth chapter deals with the logistics and distribution methods and also with the questions of organization, technical and technological solutions, with the packaging of dangerous goods just to mention the most important ones.

The book may be recommended to those who deal with packaging tasks, that is who are engaged in planning and improvement of packaging materials and systems, also in distribution and in the questions of regulation from both practical and theoretical points of view.

I. VARSÁNYI

Food Flavourings

P. R. ASHURST (Ed.)

Blackie and Son Ltd Bishopbriggs, Glasgow, 1991, 310 pages

Flavour industry is playing an important role in food and beverage industries worldwide. Food Flavourings gives a review for scientists and application technologists in the flavour and food industries.

The book contains 310 Arabic and XIV Roman numbered pages in three main parts. Chapter 1 is an introduction into the marketing of flavourings and legislative control, discusses the European Flavour Directive. The second section (chapters 2—5) covers the main groupings of raw materials — essential oils, natural extracts, fruit juices, nature-identical and artificial synthetic ingredients. The third section covers the main user industries (beverage, confectionery, bakery, dairy). This section provides an overlook of the technologies involved, where they are essential to an understanding of interaction with flavourings. In some cases they describe the development of natural flavours within the products themselves. Final chapter deals with process flavourings, becoming increasingly important. The book is intended as a source of basic information and not to provide formulation data.

M. TÓTH-MARKUS

Akadémiai Kiadó, Budapest
Acta Alimentaria 20, 1991

International Federation of Fruit Juice Producers

INTERJUICE 91

The XI International Fruit Juice Congress
to be held in Sao Paulo on 17—21 November 1991

The event which will discuss diversifications and directions for the industry in the nineties, is organized by the International Fruit Juice Federation with the support of the Brazilian associations ABRECITRUS and ANIC plus the juice producer FRUTESP, and will be attended by nearly 1000 delegates, including juice producers, bottlers, equipment and packaging manufacturers, marketing experts and scientists specializing in R + D for the sector.

INTERJUICE's program is organized so as to alternate technical sessions at the Hotel Transamerica with visits to Brazil's leading juice factories: Cargill, Citrosuco, Cutrale and Frutesp.

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Alameda Jaú, 1742-5°. andar
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PURIFICATION AND CHARACTERIZATION OF α-AMYLASE PRODUCED BY A THERMOPHILIC ISOLATE OF *BACILLUS COAGULANS*

FATMA I. EL-HAWARY

Agricultural Botany Department, Faculty of Agriculture, El-Mansoura University. Egypt

(Received: 20 September 1989; revision received: 28 June 1990; accepted: 28 June 1990)

Seven thermophilic starch hydrolyzing bacteria were isolated from soil after a preliminary enrichment in 0.6% starch broth at 55 °C. One of these isolates, identified as *Bacillus coagulans* (isolate number 75) was an active producer of α-amylase.

Three different media were used to cultivate the isolate at 55 °C with shaking at 200 r.p.m. for 48 h. Bran extract starch peptone broth (BES PB) was the most suitable medium for the production of α-amylase by *B. coagulans* 75.

The maximum production of α-amylase was exhibited at 60 °C and pH 6.0. The enzyme produced by *B. coagulans* 75 was partially purified by affinity chromatography technique up to about 78 fold. The partially purified α-amylase was active at a wide range of temperature, it remained stable and retained about 44% of its activity at 90 °C for 2 h, with half life at 85 °C for 2 h. The optimum was 5.8.

Amylolytic activity inactivated by the metal-chelating agent EDTA, and -SH group blocking agents (IAA, PCMB) and NBS.

Calcium ions play an important role in α-amylase thermostability.

Keywords: α-amylase, thermophilic starch, *Bacillus coagulans*.

Amylases are required for a number of industrial processes including brewing, paper technology, textile manufacture and saccharification of starch. The main amylolytic enzyme used is α-amylase (EC 3-2-1-1). Alpha-amylase has been isolated from several different bacilli e.g. *Bacillus stearothermophilus* (MANNING et al., 1961; BECHINA et al., 1982; SRIVASTAVA, 1984), *B. coagulans* (MAHMOUD et al., 1979; MEDDA & CHANDRA, 1980), *B. subtilus* and *B. brevis* (TSUKAGOSHI et al., 1985) and from other Bacillus spp. (SRIVASTAVA et al., 1981).

The use of enzymes with thermostability in industrial processes is of great interest. The thermostable enzymes usually have longer shelf lives and and may be easier to covalent in active form to an immobilizing support (EMI et al., 1976).

The present investigation was carried out to produce the α-amylase by a thermophilic *B. coagulans*. An attempt was made to purify and characterize the enzyme.

1. Materials and methods

1.1. Identification of bacteria

Enrichment cultures containing 0.6% starch were used with samples from soil, flour and fruits, and incubated at 55 °C for 48 h. Samples from enrichment culture were diluted and plated on agar starch medium. Colonies which had a distinct halo exceeding the colony diameter four-fold or more considered as good producers of extracellular α-amylase. These colonies were picked up, purified by several transfers on agar and designated with code numbers. Morphological, cultural and physiological properties of isolates were studied according to the routine methods recommended by the SOCIETY OF THE AMERICAN BACTERIOLOGISTS (1957). The identification was carried out with reference to BERGEY'S MANUAL OF SYSTEMATIC BACTERIOLOGY (1984).

1.2. Cultivation media

Cultivation media used in this investigation have the following composition (g dm^{-3}):

Nutrient broth (NB): Peptone 5 and meat extract 3.

Bran extract starch peptone broth (BESPB): Extract of 50 g of wheat bran; peptone, 10; KH_2PO_4, 0.3; K_2HPO_4-$3H_2O$, 0.07. The medium was prepared as described by PELTIER and BECKOR (1945), with an addition of 0.6% starch.

Potato water peptone broth (PWPB): Potato slices were boiled in water for 1 h., filtered and peptone was added to potato filtrate (TSAPLINA & LOGINOVA, 1976). The initial pH of each medium was adjusted to 6.5 ± 0.2.

1.3. Growth experiments

All experiments were carried out in 500 cm^3 Erlenmeyer flasks containing 100 cm^3 of medium. Flasks were incubated with 5% (v/v) inoculum from 24 h-old cultures grown on NB medium. Unless otherwise specified, cultures were shaken at 200 r.p.m. and 55 °C. Samples of culture broth were taken at intervals. Turbidity of the culture broth was measured at 580 nm in a Zeiss PM_4 spectrophotometer. Culture supernatant was obtained by centrifugation of culture broth at 8000 r.p.m. for 20 min.

1.4. Enzyme assay

The enzyme activity was assayed by measuring the reducing sugars liberated in the reaction mixture, the reaction mixture contained 1 cm^3 enzyme solution and 1 cm^3 1% reduced soluble starch. One unit of amylase is defined

as amount of enzyme that liberates reducing sugar equivalent to 1 μmol of maltose per min under the specified conditions. Reducing sugars were determined by the dinitrosalicylic acid method (BERGMEYER & GRASSL, 1983).

1.5. Measurement of enzyme thermostability

The enzyme solution was kept in water bath at 50, 60, 70, 80 and 90 °C. After 0.5, 1, 1.5 and 2 h, glass tubes containing samples were taken, cooled ice water and the remaining α-amylase activity was measured or described before with 0.1 mol phosphate buffer.

1.6. Purification of α-amylase

Enzyme supernatant was freeze-dried. The freeze-dried enzyme was dissolved in 0.1 mol phosphate buffer (pH 7.0) and the volume was made up to 50 cm^3. All following steps were carried out at 4 °C. Ammonium sulfate was added to the enzyme solution to give 25% saturation. The enzyme binding capacity to affinity chromatography was measured under the conditions of thermostated microcolumn (1.5×15 cm) with flow rate about 6 $cm^3\ h^{-1}$ according to HOSCHKE and co-workers (1976).

After equilibration with 25% ammonium sulfate saturation, the procedure involved binding the enzyme solution with known Sepharose 6B-cyclodextrine (CD) (VRETBLAD, 1974). Continuous washing with the same ammonium sulfate concentration was carried out to eliminate the contaminating proteins and inactive enzyme proteins until the extinction of eluted solution at 280 nm was equal to zero. The α-amylase was selectivity eluted with 0.05 mol phosphate buffer containing 0.001 mol $CaCl_2$ (pH 6.0). The active fractions were pooled after determination of the enzyme activity, dialysed against the same phosphate buffer and freeze-dried. The method of LOWRY and co-workers (1951) was used to determine the protein concentration in enzyme preparations, data were expressed as milligrams per milliliter. The enzyme activity was measured according to the method of BERGMEYER and GRASSL (1983).

1.7. Measurement of optimum pH

Measurement of optimum pH for α-amylase activity in partially purified enzyme preparations was carried out using 3 buffer solution (0.05 mol): citrate-NaOH-HCl (pH 3-7), phosphate (pH 5-7) and Tris-HCl (pH 7-9). The enzyme was diluted (1 : 1 v/v) with buffer solution and mixed with 1% starch (prepared with the same buffer solution). The α-amylase activity was measured at each pH value.

1.8. Inhibitors and activators

The effect of sulphydryl group blocking agents such as p-chloromercuric benzoate (PCMB), iodoacetic acid (IAA) (dissolved in 0.05 mol NaOH) and N-bromosuccinic acid (NBS) (dissolved in 5% isopropanol) on enzyme activity was studied. The effect of the metal-chelating agent ethylene-diamine-tetra-acetic acid (EDTA) and calcium chloride (dissolved in distilled water) was also studied.

Enzyme samples were first preincubated at 55 °C for 1 h, with inhibitors or activators diluted with 0.05 mol phosphate buffer (pH 6.0) with final concentration ranging between 10^{-1} to 10^{-3} mol, then the enzyme activity was measured. Control with the solvent alone was included.

1.9. Removal of calcium

Removal of calcium from enzyme preparations was done as described by YUTANI (1975).

2. Results and discussion

2.1. Isolation of thermophilic bacteria producing extracellular α-amylase

One isolate from seven thermophilic α-amylase producing bacteria was selected and identified after a preliminary enrichment at 55 °C in starch broth.

Results shown in Table 1 indicate that culture supernatant of isolate number 75 grown in BESPB medium exhibited the highest α-amylase activity compared with the other isolates in the same medium. Isolate number 75 was

Table 1

Effect of cultivation media on the level of amylolytic activity in culture supernatans of thermophilic bacterial isolates

Isolate No.	Cultivation media		
	BESPB	NB	PWPB
	EU cm^{-3}		
11	133	80	40
135	133	80	46
48	213	93	66
50	153	87	30
62	200	90	46
64	106	80	40
75	520	380	153

EU cm^{-3} = Enzyme activity as unit per cm^3

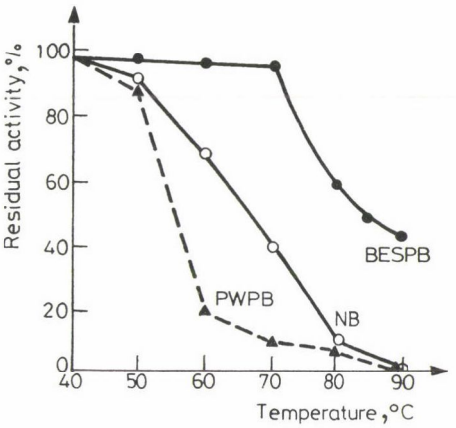

Fig. 1. Thermostability curves of α-amylase activity in culture supernatant of *B. coagulans* 75 grown in different media

therefore chosen, identified and used for further experiments. Morphological, cultural and physiological (biochemical) characteristics of this isolate were proved to be identical with those of *Bacillus coagulans* 75.

2.2. Effect of cultivation media on α-amylase thermostability

The production and thermostability of α-amylase produced by *B. coagulans* 75 was found to be affected by the composition of cultivation media as shown in Fig. 1. The highest α-amylase activity was obtained using bran extract starch peptone broth (BESPB) followed by nutrient broth (NB) and then potato water peptone broth (PWPB). The great induction of production of α-amylase by bacteria during the growth phases may be attributed to a presence of starch as substrate (SRIVASTAVA et al., 1980). MADI and co-workers (1987) found high level of amylase in Clostridium supernatant when starch was used as production substrate.

In addition, the activity of α-amylase released in BESPB medium retained about 44% of its initial activity after 2 h at 90 °C, while that elaborated in other media was completely inactivated after 2 h at the same temperature.

Alpha amylase produced in BESPB medium showed higher thermostability than that produced in other tested media. This may be due to the presence of divalent cations in BESPB medium.

2.3. Effect of incubation temperature on α-amylase production

The effect of incubation temperature on α-amylase production in BESPB medium was examined. Five different incubation temperatures, ranging between 30 °C and 80 °C, were employed. Results shown in Fig. 2 indicate that

raising the incubation temperature increases the enzyme production up to a maximum of 60 °C. Beyond this temperature, production of α-amylase by *B. coagulans* 75 decreased.

It should be emphasized that the optimum temperature may depend on the substrate (VAN DER ZANT, 1957). MEDDA & CHANDRA (1980) and GRUENINGER and co-workers (1984) reported that temperature optima of α-amylase production (60–70 °C) were frequently higher than the maximum temperature (50–60 °C) of bacterial growth.

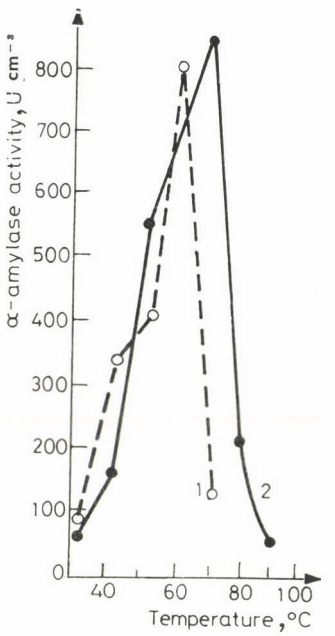

Fig. 2. Effect of temperature on enzyme production (1) and enzyme activity (2)

2.4. *Enzyme purification*

Culture supernatant was prepared as previously described in Materials and methods. The enzyme was eluted in a single peak using 0.05 mol phosphate buffer (pH 6.0) on Sepharose 6B-CD column. The purification steps are summarized in Table 2. A 78-fold increase in α-amylase activity was obtained with 67% recovery. Almost half of the enzyme activity and thermostability recovery was lost during purification steps.

Table 2

Summary of enzyme purification steps

Purification steps	Enzyme solution (cm^3)	Total protein content (mg)	Total activity (units)	Specific activity fold (unit mg^{-1})	Purific action (fold)	Recovery (%)
Culture supernatant	200	11.550	43410	3.75	1	100
Lyophilized	50	11.550	43410	3.75	1	100
Sepharose GB CD	6	100	29240	292.4	78	67

2.5. *Optimum pH*

The maximum activity of the partially purified α-amylase was exhibited at pH 5.8 in 0.05 mol phosphate buffer (Fig. 4). The activity declined sharply on the other sides of the curve, with no detectable activity over pH 8.0. These results are in accordance with those obtained by BOVA (1982) who reported that α-amylase of *B. coagulans* had optimum pH of 5.8, and BURANAKARL and co-workers (1988), who found that optimum pH was 6.0 for the enzyme produced by photosynthetic bacteria.

On the other hand UPTON and FOGARTY (1977) and MAHMOUD and co-workers (1979) reported that α-amylase of *B. coagulans* and *B. stearothermophilus* had optimum activity at pH 7.5. SRIVASTAVA and co-workers (1984) ound that optimum pH for many species of Bacillus was 6.5.

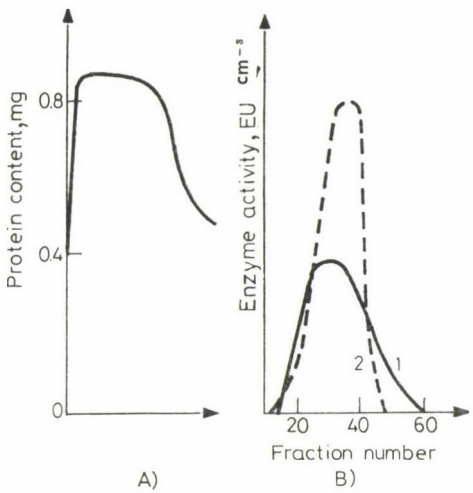

Fig. 3. Elution of α-amylase enzyme on Sepharose 6B-CD column with flow rate 6 h. A.: Eluted by ammonium sulphate at 25%, protein estimated at 280 nm. B: Eluted by phosphate buffer 0.01 mol at pH 6.0, enzyme activity estimated at 540 nm. 1: protein content; 2: enzyme activity

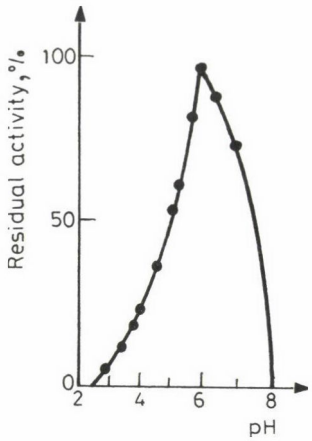

Fig. 4. Effect of pH on α-amylase activity of *B. coagulans* 75

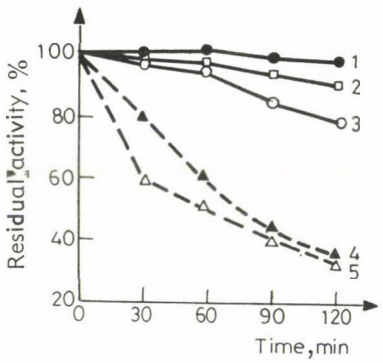

Fig. 5. Thermostability of α-amylase produced by *B. coagulans* 75. 1: 50 °C; 2: 60 °C; 3: 70 °C; 4: 80 °C; 5: 90 °C

2.6. Effect of temperature on the enzyme activity and stability

The optimum temperature for α-amylase activity produced by *B. coagulans* was found to be 70 °C as shown in Fig. 1. These results are in agreement with those obtained by BECHINA and co-workers (1982) and ANTRANIKIAN and co-workers (1987) but different from those of TSVETKOV and EMANUILOVA (1989), who dealed with α-amylase produced by *B. brevis* at 80 °C.

The enzyme is completely stable at 50 °C for 1 h while 95% and 32% of the residual activity was retained at 60 °C and 90 °C for 1 h, respectively (Fig. 5). The α-amylase showed no activity at 100 °C. The half-life of partially purified α-amylase at 85 °C was found to be 2 h. These thermostable characteristics were investigated for α-amylase of *B. brevis* and *B. licheniformis* by MORGAN and PRIEST (1981) and TSVETKOV and EMANUILOVA (1989), they found that the produced enzyme was stable at 90 °C for 20 min.

2.7. Effect of inhibitors

As shown in Table 3, the enzyme activity is strongly inhibited by all sulfhydryl group blocking agents (IAA and PCMB). The enzyme restored only 40% and 36% of its activity after treatment with 10^{-1} mol of IAA and PCMB, respectively. The loss of the enzyme activity indicates that the sulfhydryl group is essential for amylolytic activity (SRIVASTAVA, 1984).

Table 3

Effect of sulfhydryl group blocking agents, EDTA, Ca^{++} and Ca^{++} + EDTA on activity of α-amylase produced by B. coagulans

Agents	Concentration (mol)	Relative activity (%)
Control		100
IAA	10^{-1}	40
	10^{-2}	59
	10^{-3}	78
PCMB	10^{-1}	36
	10^{-2}	49
	10^{-3}	76
NBS	10^{-1}	55
	10^{-2}	75
	10^{-3}	92
EDTA	10^{-1}	62
	10^{-2}	75
	10^{-3}	88
Ca^{++}	10^{-1}	100
	10^{-2}	100
	10^{-3}	100
Ca^{++} + EDTA	10^{-1}	92
	10^{-2}	92
	10^{-3}	98

The enzyme activity was inhibited by (NBS) as shown in Table 3. It is known that NBS is not a specific reagent (SPANDE & WITKOP 1967).

Modification with NBS reagent occurs by cleaving tryptophyl peptide bonds resulting in an enzyme inactivation without a detectable change in amino acid composition.

On the other hand, the investigation by the metal chelating agent (EDTA) shows a possible dependence on bivalent cations, which binds the subunits of enzyme (Table 3). The experiments concerning the substitution of Ca^{++} and (Ca^{++} + EDTA) showed that Ca^{++} is indeed essential for full enzyme activity (Table 3) (VALLEE et al. 1959; FISCHER & STEIN 1960; YUTANI 1975).

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PECTOLYTIC ENZYMES IN PRODUCING MANGO JUICE

J. K. GITHAITI and E. G. KARURI[a]

Department of Food Technology and Nutrition, University of Nairobi, P.O.Box 29053, Nairobi. Kenya

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Commercial pectolytic enzyme preparations from various sources exhibited different modes of activity when used on a mango mash substrate. At a specific temperature and dose, two types of reaction were observed. One type of reaction resulted in rapid viscosity drop, but the products were unstable on standing. The other type showed an initial viscosity rise followed by a gradual drop. The latter pulps were cloud-stable on standing and quite viscous. To improve the cloud-stability of the concentrate prepared from the liquefying enzymes, the concentrate was cutback with the macerated pulp. Yield was improved in both treatments but was more pronounced with the macerating enzymes.

Keywords: pectolytic enzymes, mango juice production, liquefying enzymes

Mango (*Mangifera Indica* L.) has become an important commercial fruit in Kenya. Improved fruit cultivars are grown and exported as fresh fruit to the Middle East and European markets. Such include the Ngowe, Boribo, Peach and the Tommy Atkins, which are non-fibrous and have an attractive appearance. Fibrous varieties are either wasted in the field, consumed locally or to a lesser extent made into nectars. Production of juice concentrates and other products would help preserve the seasonal mango fruit and open new channels for use of the fruit, commercially (SHARMA, 1981; GACHANJA, 1985).

Various methods have been used in mango juice extraction but these have resulted in low yields (BREKKE et al., 1975; GATCHALIAN et al., 1975). An effort has also been made to produce mango juice concentrates but this has not been successful due to the high viscosity inherent in mango juice (ASKAR et al., 1981). Cloud stability and appropriate viscosity in some fruits can be achieved by a short blanching followed by comminution and homogenization. Achieving the required physical characteristics in some tropical and subtropical fruits requires a different approach as the fruits often deteriorate before the right state is achieved. Use of biochemical methods in production of cloudy, free-flowing natural flavoured single strength juices and concentrates has gained considerable importance in fruits grown under temperate climatic

[a] To whom all the correspondence should be addressed.

conditions. An extension of similar work on tropical and subtropical fruits would open up a new area of novel fruit juices (BERTUZZI, 1961; LUH, 1971; GANTER, 1972; SCHOBINGER, 1980).

This investigation was aimed at optimizing enzymatic treatment of mango mash to improve yields and obtain a product of low viscosity suitable for concentration and still remaining cloud stable.

1. Materials and methods

Ready to eat ripe mangoes were obtained from commercial centres in Nairobi and Kenya Horticulture Exporters (KHE). The nonfibrous Ngowe and fibrous local (Machakos) varieties were used.

The enzymes evaluated were: Ultrazym 100G, Ultrazym M10, Pectinex 3XL (Novo Ferment Basel Switzerland) and Rohapect TF, Rohament PC (Rohm Enzyme Technology, Darmstadt, FRG).

About 6–10 kg of mangoes were blanched in steam for 2 min. The fleshy part was then cut from the stone with knife before being disintegrated by a laboratory fruit blender. The mash together with the stones were heated to 40 °C and maintained at that temperature for enzyme treatment. A dose of 200 ppm enzyme was used of all enzymes with the two mango varieties. Samples of 1 kg were withdrawn at 30 min intervals and the juice pressed out by hand through a muslin press cloth. Yield, viscosity and cloud stability were evaluated in this juice. Yield was reported as a percentage of the whole fruit mash by weight. Viscosity was determined at 20 °C using a rotating viscometer (STV Rheomat Contraves, Zürich, Switzerland).

Table 1

Changes in yield and viscosity of the enzyme-

Time (min)	Enzyme/Mango variety					
	A		B		C	
	Yield (%)	Viscosity (Pa s)	Yield (%)	Viscosity (Pa s)	Yield (%)	Viscosity (Pa s)
0	51.1	0.655	59.5	0.162	47.2	0.399
30	68.9	0.859	69.9	0.318	67.3	0.845
60	71.0	0.986	72.5	0.407	70.4	0.739
90	72.0	0.908	73.2	0.410	71.9	0.641
120	72.8	0.850	73.7	0.172	71.1	0.602
150	73.0	0.775	74.0	0.157	71.2	0.592

A: Ultrazyme M10 (Local); B: Ultrazyme M10 (Ngowe); C: Rohament PC (local); D: Ultrazyme 100G (local); E: Ultrazyme 100G (Ngowe); F: Rohapect TF (local)

Cloud stability was established by separation of the pulp into a clear supernatant and sediment on standing. Mango mash maintained under the same conditions but without enzyme treatment was used as a control.

2. Results

Table 1 and Figs. 1, 2 and 3, summarise the effects of treatment of various commercial enzyme preparations on mango mash.

A general improvement on pulp yield was observed with practically all enzymes tested at a rate of 0.02% (200 ppm), compared to the untreated mash. In nearly all cases, yields were increased by more than 10%. Figure 1 shows the changes in yield with treatment time. There was a gradual increase in yield up to 60 min when a near constant yield was attained. This resulted due to enzymatic breakdown of the pectin in the mango mash. Breaking down of soluble pectin, disintegrating fruit tissue into cell aggregates and individual cells and releasing cell contents by opening up the cell wall, results in improved yields (PILNIK & ROMBOUTS, 1979).

The specific composition of the two varieties is seen to affect the pulp yields. This is due to the ratio of stone to pulp in both varieties, which is higher in Ngowe than in the Local (Machakos) variety.

Two distinct modes of viscosity change and nature of product cloud stability were observed. Ultrazym M10 and Rohament PC caused an initial rise in viscosity followed with time by a gradual fall (Fig. 2). Products from these treatments were quite viscous and retained a stable cloud. Ultrazym 100G, Pectinex 3XL and Rohapect TF treatment resulted in rapid viscosity

treated mango mash in dependence of treatment time

Time min	Enzyme/Mango variety					
	D		E		F	
	Yield (%)	Viscosity (Pa s)	Yield (%)	Viscosity (Pa s)	Yield (%)	Viscosity (Pa s)
0	53.2	0.458	59.5	0.162	47.2	0.399
30	61.8	0.015	68.3	0.017	62.6	0.029
60	67.5	0.006	69.2	0.014	63.9	0.024
90	67.8	0.006	70.8	0.005	64.1	0.010
120	68.1	0.006	71.6	0.004	64.3	0.007
150	68.9	0.006	72.4	0.004	64.4	0.006

Enzyme dose: 200 ppm
Temperature: 40 °C

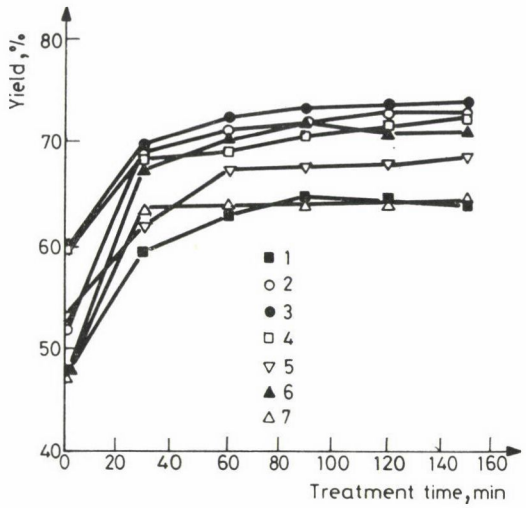

Fig. 1. Variation of yield with treatment time for five enzymes; 1: Pectinex 3XL (local), 2: Ultrazyme M10 (local), 3: Ultrazyme M10 (Ngowe), 4: Ultrazyme 100G (Ngowe), 5: Ultrazyme 100G (local), 6: Rohament PC (local), 7: Rohapect TF (local)

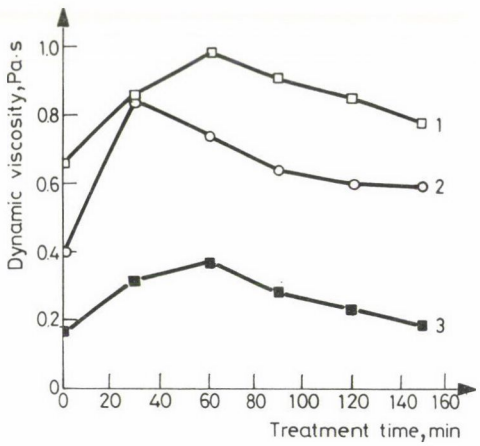

Fig. 2. Changes in mango pulp viscosity during treatment with macerating enzymes; 1: Ultrazyme M10 (local), 2: Rohament PC (local), 3: Ultrazyme M10 (Ngowe)

drop with time (Fig. 3). These low viscosity products were not cloud-stable. A viscosity drop of over 85% was observed with Ultrazym 100G compared with 82% reported by SREENATH and co-workers (1987). Yields were observed to be higher with the first set of enzymes than with the second set.

These observations can be explained as due to the specific composition of the different enzyme preparations. Based on the proportions of pectin

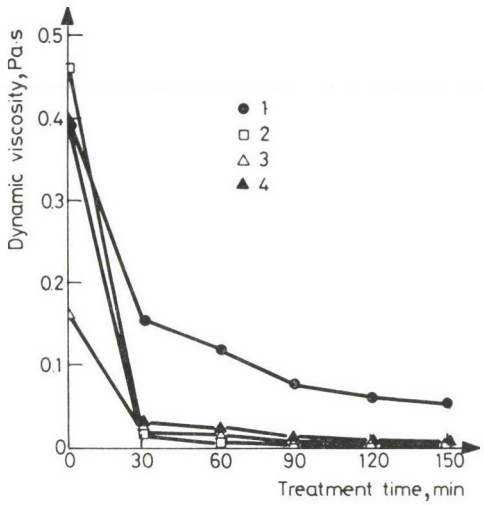

Fig. 3. Changes in mango pulp viscosity during treatment with liquefying enzymes; 1: Pectinex 3XL (local), 2: Ultrazyme 100G (local), 3: Ultrazyme 100G (Ngowe), 4: Rohapect TF (local)

esterase (PE), polygalacturonase (PG), pectate methyl lyase (PML) and pectin lyase (PL) in each formulation, enzymes can be applied for either clarification, liquefaction or maceration. Enzyme preparations with high amounts of PE together with PG, PL and PML result in rapid viscosity drop and loss of cloud stability. Ultrazym 100G, Pectinex 3XL and Rohapect TF can be said to have such composition. Enzymes with high amounts of PG, PL and PML but little or no PE will result in viscous products which are cloud-stable (GRAMPP, 1972). These are referred to as macerating enzymes. Ultrazym M10 and Rohament PC may have such a composition.

The initial viscosity increase in the macerated pulps would be due to an initial solubilization of pectin from fruit tissue resulting in a viscosity rise. In this case the rate of solubilization is higher than the rate of pectin breakdown. This is followed by a stage of hydrolysis (breakdown), which overtake the rate of solubilization causing a gradual viscosity drop (BAUMANN, 1979).

The above results indicate that pectolytic enzymes would be of great importance in improving mango pulp yields and producing pulp viscosities suitable for concentration. However, macerating emzymes produce pulps of high viscosities which therefore cannot be concentrated to high levels. Cloud-stable mango juice concentrates can therefore be prepared only by the "cut-back" method like in citrus juice (BERK, 1968; PALA & BIELING, 1978).

3. Conclusions

Pectolytic enzymes may be used to improve the yield of mango juice and also to facilitate the manufacture of mango concentrate. With the cutback technique, cloud-stable concentrates may be produced.

At the selected enzyme dose of 200 ppm and a temperature of 40 °C, the maximum change in yield and viscosity occurred in 60 min.

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STUDIES OF THE OIL OF *MIMUSOPS ELENGI* SEED

B. MANDAL[a] and C. R. MAITY[b]

[a]Department of Chemistry, B. N. Mahavidyalaya, Itachuna-712147, Hooghly, W. B. India

[b]Department of Biochemistry, Burdwan Medical College, Burdwan-713104, W. B. India

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Extraction of *Mimusops elengi* seed kernels yielded 22.4% oil. The crude oil was refined with alcohol–alkali solution and bleached with activated earth and carbon. The fatty acid composition (wt%) of the refined seed oil was: palmitic 10.7, stearic 10.2, behenic 0.5, oleic 64.1 and linoleic 14.5, acid %, respectively. Nutritional evaluation of the refined seed oil was carried out in rats by feeding the seed oil and groundnut oil as control at 10% level in a 10% protein diet for 4 weeks. Nutritional quality of the refined seed oil as judged by growth performance, digestibility, feed efficiency ratio, serum lipid chemistry and histopathological findings was satisfactory and well comparable to that of groundnut oil.

Keywords: *Mimusops elengi*, unconventional plant seed, fatty acid composition, nutritional evaluation

We have started a systematic study in our laboratory to search for newer and unconventional sources of oil and as a part of this study, the chemical and nutritional characteristics of the seed oil of *Mimusops elengi* Linn. of Sapotaceae family are reported here.

Mimusops elengi is large handsome evergreen tree abundantly found in local forests and roadsides of India. A large tree can produce about 50–1000 kg seeds annually. The seeds are at present discarded as waste material.

1. Materials and methods

The ripe fruits were collected and seeds were removed from air-dried fruits. The dried seed kernels were powdered and extracted with n-hexane in a Soxhlet distillation apparatus for 72 h. The solvent was removed in a rotary evaporator at 35–40 °C.

1.1. Processing of the seed oil

The crude oil was first filtered and then refined by using alcoholic caustic soda in three successive stages of concentrations of 0.3, 0.2 and 0.1%, respectively. The refined oil was bleached with 2% activated fuller's earth and 0.2% carbon.

1.2. Analytical methods

The refined seed oil was analysed for physicochemical characteristics by the AMERICAN OIL CHEMISTS' SOCIETY (1973) methods. Methyl esters of the refined oil were prepared by transesterification of the oil using sodium methoxid in methanol after diazomethane esterification of the free fatty acids. The methyl esters were used for determining fatty acid composition by gas liquid chromatography (GLC) using a 15% DEGS column on chromosorb WHDS (MAITY & MANDAL, 1990). Infrared (IR) spectra of the oil and its methyl esters were taken using Beckman Model 221 IR spectrophotometer in KBr disc and ultraviolet (UV) absorption was taken in CCl_4 on a Beckman 26 UV-visible spectrophotometer. Thin-layer chromatography (TLC) of the oil and its methyl esters was done separately on 0.25 mm Silica Gel G coated glass plates using n-hexane, diethyl ether and acetic acid (79 : 20 : 1) and spraying with concentrated sulphuric acid. The methyl esters of castor and sal oils were used for reference.

1.3. Nutritional evaluation

Twelve male albino rats of local strain (inbred in our laboratory) weighing about 50–60 g were divided into two groups of 6 animals each and individually caged. Animals in each group were fed a 10% protein standard diet containing 10% oil (MANDAL et al., 1982). One group was used for groundnut oil and another for refined seed oil. The animals received their respective diet and water ad libitum for 4 weeks. Food intakes were recorded daily and body weight twice weekly. Feed efficiency ratio (FER) which represents the weight gain for unit food intake was calculated. Digestibility of the oil was determined by estimating the oil intake and oil excreted through urine and faeces (RAGHURAMULU et al., 1983). At the end of 4 weeks, the animals were sacrificed, blood was collected and total lipids (FOLCH et al., 1957), phospholipids (FISKE & SUBBAROW, 1925), free fatty acids (HEINDEL et al , 1974) and total cholesterol (SPERRY & WEBB, 1950) were determined. Organs like liver, heart, kidney and, reproductive organs were subjected to histopathological examination under microscope.

2. Results

The seed kernels yielded 22.4% oil. The crude oil was reddish brown in colour with an unpleasant odour. Alcohol–alkali refined and bleached oil was almost colourless and odourless. The refined product formed 90% of the crude oil. The UV and IR spectra did not indicate the presence of conjugation or trans unsaturation, respectively. The TLC analysis showed the absence of any oxygenated or cyclopropene fatty acids in the oil. The physicochemical charac-

teristics of the refined seed oil were normal (Table 1). The results show that oleic acid is the major fatty acid amounting to nearly 64% followed by linoleic acid (14.5%), stearic acid (10.2%), palmitic acid (10.7%) and a trace amount of behenic acid (0.5%). The results of the feeding study are shown in Table 2. It has been found that the rats fed diet containing 10% refined seed oil showed body mass gain which was almost similar to that obtained with groundnut oil.

Table 1

Physiochemical characteristics and fatty acid composition of the refined seed oil of Mimusops elengi

Physicochemical constants:	
Refractive index at 25 °C	1.4658
Saponification value	187
Iodine value	81.4
Unsaponifiable matter (%)	1.4
Acid value	2.8
Fatty acids (wt%):	
Palmitic	10.7
Stearic	10.2
Behenic	0.5
Oleic	64.1
Linoleic	14.5

The data are mean value of three determinations

Table 2

Nutritional indices of rats fed diets containing groundnut oil and the refined seed oil of Mimusops elengi *for 4 weeks*

Parameters studied	Diet containing 10% groundnut oil		Diet containing 10% refined seed oil	
	$\bar{x}$	± s	$\bar{x}$	± s
Body mass gain (g) for 4 weeks	48.3	4.6	46.9	5.1
FER	24.3	2.4	22.8	2.3
Digestibility of fat (%)	94		92	
Serum:				
Total lipids (mg per 1000 cm^3)	1254	82	1296	78
Phospholipids (mg per 1000 cm^3)	782	36	749	34
Cholesterol (mg per 1000 cm^3)	628	43	683	38
FFA (mmol per 1000 cm^3)	0.32	0.02	0.34	0.05

$\bar{x}$ = mean value from six rats; ± s = standard deviation
FER = feed efficiency ratio = body mass gain/food intake ×100
FFA = free fatty acids
All value are non-significant when compared between the two groups

The mean value for FER of the refined seed oil was 22.8, a figure which was very close to that seen with groundnut oil. Digestibility of the seed oil was 92% compared to 94% for groundnut oil. Serum lipid parameters of the rats fed seed oil were normal and compared well to those fed groundnut oil. No histopathological abnormalities were found in any organ of rats fed either the refined seed oil or groundnut oil.

3. Conclusions

3.1. Chemical characteristics

High oil content (22.4%) in *M. elengi* seed kernels suggest the possibility of its commercial exploitation. The crude oil has a reddish brown colour and an unpleasant odour, high unsaponifiable matter and high acid value. The alcohol–alkali refining and bleaching reduce the visible colour of the seed oil and also reduce the unsaponifiable matter from 8.3% to 1.4% and acid value from 20.1 to 2.8. Loss on refining was also very low (10%). The physicochemical characteristics and fatty acid composition of the refined oil are similar to those of some common edible oils. Toxic non-glyceride components are not found by chemical analysis and the refined seed oil is devoid of oxygenated (epoxy or hydroxy) and cyclopropene fatty acids. The refined oil acquires a yellow tint on storage and the free fatty acid content increases from 2.8% to 6.8% during storage for one year. These hazards could be minimized on storage of the oil without the exposure to air.

3.2. Possibility of the refined seed oil for nutritional exploitation

The results of nutritional evaluation show that the rats fed 10% seed oil for 4 weeks gained a body weight which closely resembles that obtained with groundnut oil. The value for FER and the digestibility of the refined seed oil are similar to that seen with groundnut oil. There are, however, no significant differences in the concentrations of serum lipids in either of the two groups. Histopathological examination does not reveal any abnormalities in any organ of the rats fed the seed oil. Thus, the nutritional qualities of the refined seed oil is satisfactory and comparable to that of groundnut oil. Therefore, *M. elengi* seed appears promising for use as a source of dietary fat.

*

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INVESTIGATION OF THE SOLUBILITY OF CEREAL GERM PROTEIN CONCENTRATES

E. A. HEBSHI[a]

Department of Food Technology Higher Institute of Technology, Brack. Libya

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The solubility of cereal (wheat, rice, barley and maize) germ protein concentrates was investigated at different pH values (pH 2–10) and temperatures (30–70 °C). High solubility was observed at higher pH values and low solubility was measured in the pH range 4 to 6. In strongly acidic media (pH 2) the solubility was moderate. The solubility increased with increasing temperature till the beginning of denaturation. The temperature of maximum solubility is between 50–70 °C depending on the protein quality and pH.

Keywords: germ protein, protein solubility, cereal germ

It is assumed that the solubility of proteins is the property which affects their functionality and application (RUTKOWSKI & GWIAZDA 1986; LÁSZTITY, 1989). From a practical point of view, data on solubility characteristics are very useful for determining optimum conditions for extraction and purification of proteins from natural sources, and for the separation of protein fractions. Solubility behaviour under various conditions also provides a good index of the potential applications of proteins.

This is so because the degree of insolubility is probably the most practical measure of protein denaturation + aggregation, and because proteins that initially exist in a denaturated, partially aggregated state often exhibit impaired ability to participate effectively in gelations, emulsifications, and foaming. Finally, solubility is also an important attribute of proteins selected for use in food beverages.

Solubility of most proteins is markedly and irreversibly reduced when heating is involved. Nevertheless, heat treatment is needed to achieve other objectives such as microbial inactivation, removal of off-flavours, removal of water, and others (FENNEMA, 1985).

Among the new protein sources which may be used for direct human consumption cereal germ proteins may play a significant role. Cereal germs are long known for their high protein content, minerals and vitamins. Cereal germs can be used as valuable nutrient fortifiers in both food and feed (TSEN,

[a]Present address: Department of Biochemistry and Food Technology, Technical University Budapest, H-1502, Pf. 91. Budapest. Hungary

1980; CERLETTI & RESTANI, 1985). After defatting the proteins of germ may be extracted and protein concentrates and isolates can be produced (RESTANI et al., 1980).

The purpose of investigations summarized in this paper was the enlargment of our knowledge concerning functional properties of cereal germ proteins. For comparison a soy concentrate and vital gluten was also investigated.

1. Materials and methods

Wheat, maize and rice germ was obtained from State Farm Monor (Hungary). All germs were produced by dry-milling process, transported after separation to our laboratory and stored at 4–6 °C. Malted barley germ (from Dreher Brewery, Budapest) was also used. All germs were ground and defatted by n-hexane before extraction of protein. Wheat and rice germ proteins and malted barley germ protein were isolated applying twofold 3% NaCl solution extraction. Maize germ protein was isolated by the method used by NILSEN and co-workers (1973). Soy concentrate (Purina) and vital gluten (Wheat Starch Factory, Budapest, Hungary) were also used in the experiments. The protein content of preparations is shown in Table 1.

Table 1

Gross chemical composition of protein preparations used in experiments

Sample	Protein (%)	Moisture (%)	Lipid (%)	Ash (%)
Purina 500 E	89.6	5.1	0.8	3.6
Vital gluten	80.0	5.0	0.1	2.0
Wheat germ protein concentrate	75.0	6.1	3.5	2.1
Rice germ protein concentrate	72.0	5.8	3.2	1.8
Maize germ protein concentrate	71.5	5.2	8.2	2.3
Malted barley germ protein concentrate	78.2	6.2	2.1	3.8

The solubilities of the different protein samples were examined as a function of pH and temperature, by the method described by WANG and KINSELLA (1976). Protein samples were suspended in water (50 mg 5 cm^{-3}). The pH of each suspension was adjusted to the desired pH with 1 N NaOH or/and 1 *N* HCl (pH 2–10). The samples then were shaken in a water bath of the desirable temperature (30–70 °C). An aliquot part of each protein suspension was filtered. The protein in the fluid was quantitated by the photometric method of LOWRY and co-workers (1951).

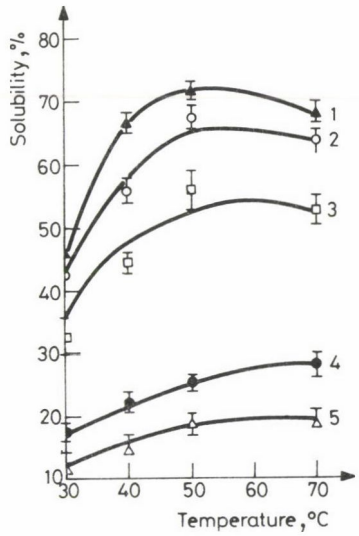

Fig. 1. Solubility of soy protein isolate at different temperatures and pH-s
1: pH 10; 2: pH 8; 3: pH 6; 4: pH 2; 5: pH 4

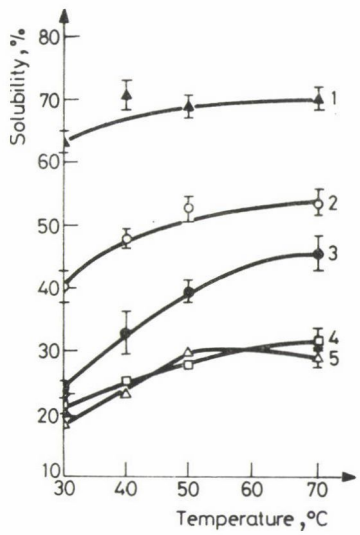

Fig. 2. Solubility of rice germ protein concentrate at different temperatures and pH-s
1: pH 10; 2: pH 8; 3: pH 2; 4: pH 6; 5: pH 4

2. Results and discussion

Figures 1–6 show the solubility of the different protein samples as a function of temperature at different pH values.

Most of the solubility increases take place between 30 and 50 °C. Over 50 °C, the rate of increase in the solubility decreases or even the rate of solu-

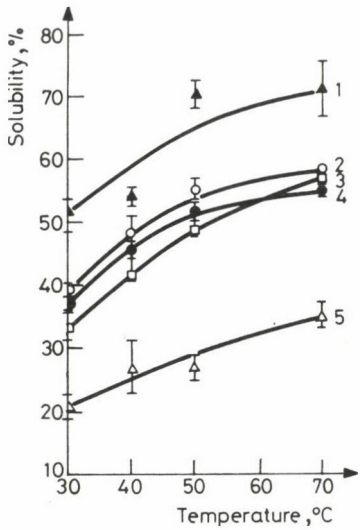

Fig. 3. Solubility of vital gluten at different temperatures and pH-s
1: pH 10; 2: pH 8; 3: pH 6; 4: pH 2; 5: pH 4

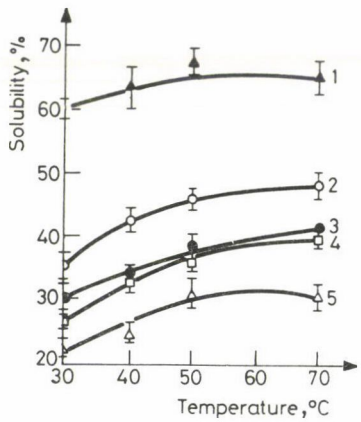

Fig. 4. Solubility of wheat germ protein concentrates at different temperatures and pH-s
1: pH 10; 2: pH 8; 3: pH 2; 4: pH 6; 5: pH 4

bility decreases. The solubility of the different protein samples at the different temperatures is high at high pH values (pH 10), low in pH value range 4–5 (near the isoelectric point) and moderate with very low pH s. (pH 2). Soy and malted barley protein solubility increase rapidly between 30 to 45 °C and starts to decrease from 50 °C to 70 °C which may be due to the beginning of denaturation of the proteins. The solubility of wheat- and maize germ protein concentrates increases slowly from 30 to 60 °C and then very slowly to 70 °C. The solubility of gluten and rice germ protein concentrate increases rapidly from 30 °C to 50 °C and then slowly to 70 °C.

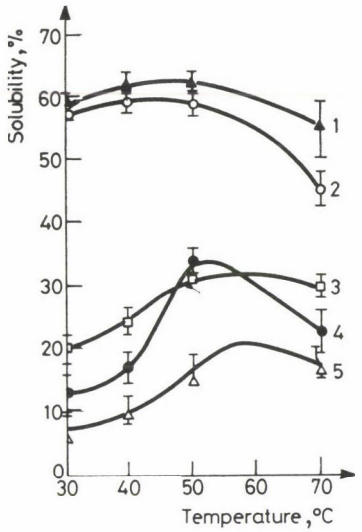

Fig. 5. Solubility of malted barley germ protein concentrate at different pH-s and temperatures
1: pH 10; 2: pH 8; 3: pH 6; 4: pH 2; 5: pH 4

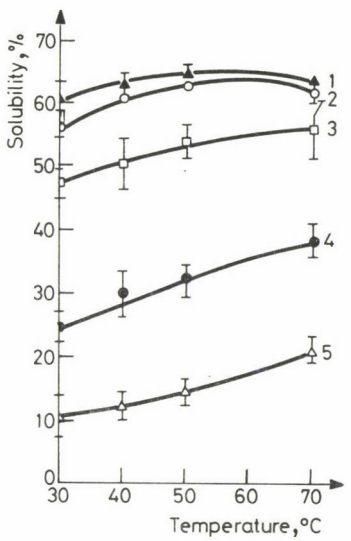

Fig. 6. Solubility of maize germ protein concentrate at different temperatures and pH-s
1: pH 10; 2: pH 8; 3: pH 6; 4: pH 2; 5: pH 4

The solubilities of the six protein samples at different pH values showed appreciably different temperature dependence. It was found that the maximum is reached between 45 ° and 60 °C for most of the protein samples and the minimum at either lower or higher temperatures. These results may be very useful in extraction and purification of proteins, by adjusting the optimum temperature and pH and high solubility can be achieved. Results are of interest also for producers of protein fortified food products, particularly protein beverages.

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HPLC-ANALYSIS OF CAROTENOIDS IN IRRADIATED AND ETHYLENE OXIDE TREATED RED PEPPER

GY. ZACHARIEV[a], I. KISS[a], J. SZABOLCS[b], GY. TÓTH[b], P. MOLNÁR[b] and Z. MATUS[b]

[a]Central Food Research Institute, H-1525 Budapest, P. O. Box 393. Hungary
[b]Institute of Chemistry, University Medical School, H-7643 Pécs, Szigeti út 12. Hungary

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The effect of two microbial count reducing treatments — irradiation and fumigation with ethylene oxide — on the content of carotenoids in red pepper, were compared.

By HPLC-technique the percentage ratio of 11 carotenoids in the total carotenoid content could be determined quantitatively. The changes in the percentage ratio of the different carotenoids caused by the two treatments were statistically significant but from the practical point of view the changes were negligible.

It was proved by HPLC technique that the carotenoids of irradiated paprika are as stable as those of the ethylene oxide treated paprika sample.

Keywords: HPLC-analysis, carotenoids, irradiation, ethylene oxid treatment, red pepper

The total viable cell count of ground paprika varies generally between 10^4–10^6 g^{-1} and the mould count may approach the value of 10^3 g^{-1} (KISS & FARKAS, 1988). This high microbiol load does not meet the strict hygienic requirements which prevail nowadays over the food market of the world.

Treatment with ethylene oxide, introduced for spices by CORETTI in 1957 has become the most widely used method of decontamination on a commercial scale. Ethylene oxide, however, reacting with inorganic chlorides forms ethylene chlorohydrin in foods, a persistent toxic substance. Considering this its application has been prohibited in several countries. In Hungary commercial scale ethylene oxide treatment is performed only if it is a definite wish of the buyer firm. The Research Institute for Canning and Paprika Industry used to control the ethylene chlorohydrin content of fumigated paprika batches before export (VIDÁCS-LICHTNER, 1982).

During the last three decades ionizing radiation has proved to be an efficient alternative to gas fumigation which does not involve the formation of toxic residues.

SZABAD and KISS (1979) found that in the case of ground paprika the cell count reducing effect of a 5 kGy dose of gamma radiation was equivalent to that of a commercially applied ethylene oxide treatment. The gas treatment

did not affect the mould count, whilst in the irradiated paprika samples moulds were not detectable.

In other study it was established that sanitizing irradiation did not reduce either the pigment or the capsaicin content of paprika (FARKAS et al., 1973).

The pigment of paprika is composed of carotenoids. Purified carotenoid compounds proved to be very sensitive to gamma-irradiation when dissolved in organic solvents or emulsified in water (ZACHARIEV & KISS, 1983). The stability of these carotenoids increased considerably when they were adsorbed onto a solid support (ZACHARIEV & KISS, 1984).

The sensitivity of carotenoids to chemical agents is also known from the literature (ZECHMEISTER, 1962). Therefore it seemed relevant to study in a comparative analysis the influence of gamma irradiation and ethylene oxide treatment. Instead of the so-called total pigment content, a value based on the gross absorbance of the benzene extractable substances, the finer carotenoid pattern was analysed by HPLC technique.

1. Materials and methods

"Extra" quality ground paprika used in the experiments was grown around Szeged and homogenized and put at our disposal by the Paprika Processing Plant, Szeged.

1.1. Irradiation

Radiation treatment was carried out in the RH-gamma-30 type ^{60}Co self-shielded radiation source at ambient temperature under aerobic conditions. Ground paprika samples were irradiated with a 5 kGy dose.

1.2. Treatment with ethylene oxide

Fumigation was carried out at the Paprika Processing Plant, Szeged. The samples were exposed, in a Degesch-type equipment for 6 h at 30–35 °C, to 0.64 kg m^{-3} gas concentration. Beside the experimental samples 5 tons of paprika were treated simultaneously in the equipment.

Ground paprika was dried for 4.5 h at 95 $\pm$ 2 °C to determine the dry matter content and for solvent extraction.

1.3. Determination of the carotenoid composition by HPLC

The dried ground paprika was extracted with benzene. An aliquot of the benzene extract was evaporated then dissolved in ether. The carotenoid esters present were saponified with a methanolic KOH solution in heterogeneous

phase. After separating the alkaline phase the solution was repeatedly extracted with ether. The united ethereal extracts were washed with water free of alkali and methanol then dried over anhydrous Na_2SO_4. The mixture of carotenoids thus obtained, was used for the determination of Benedek-Number as well as for high-performance liquid chromatography (HPLC). Benedek-Number was determined according to HUNGARIAN STANDARD (1976).

In the knowledge of the Benedek-Number an aliquot of the saponified carotenoid mixture was transferred to acetone to give a solution of 100–200 $\mu g\ cm^{-3}$ concentration. For quantitative analysis to an aliquot of the sample, depending on its pigment content, an amount of cantaxanthin stock-solution was added as an internal standard.

Measurements were carried out with a Liquochrom Model 307 liquid chromatograph (Labor MIM, Hungary) with a Beckmann UV-Vis spectrophotometric detector adapted for this purpose, and a mechanic integrator made by Carl Zeiss, Jena using a home-packed column.

Conditions of chromatography:
Column: Chromsil-C_{18} 10 μm (250×4.6 mm; Labor MIM)
Eluent: 100 : 38 (v/v) acetone–water then changed to 100 : 5
Flow rate: 2.50 $cm^3\ min^{-1}$
Sample: 10–15 μl
Detection wavelength: 480 nm
Detection sensitivity: 0.16 A

Individual peaks in the chromatograms were identified according to MATUS and co-workers (1981) by means of retention times of authentic samples or by modifying reactions causing changes in retention times (acidification for epoxide containing carotenoids; reduction for compounds with oxo-group).

Three separate saponifications were carried out of every samples. Two parallel chromatograms were used for the quantitative evaluation of each saponified sample. About 91–93% of the carotenoids were contained in 6 peaks for each of which calibration was made with model substances. An alternative method of high-performance liquid chromatography omitting saponification was elaborated by CS. PAVISA and co-workers (1985).

2. Results

2.1. Effect of treatments and storage time upon the carotenoid composition of the pigment of ground paprika

Figure 1 shows the chromatograms of untreated, irradiated and ethylene-oxide-treated ground paprika stored for 4 months.

Numbers increasing from right to left mark the following carotenoids: capsorubin (1); capsanthin-epoxide (2); violaxanthin (3); a partly separated

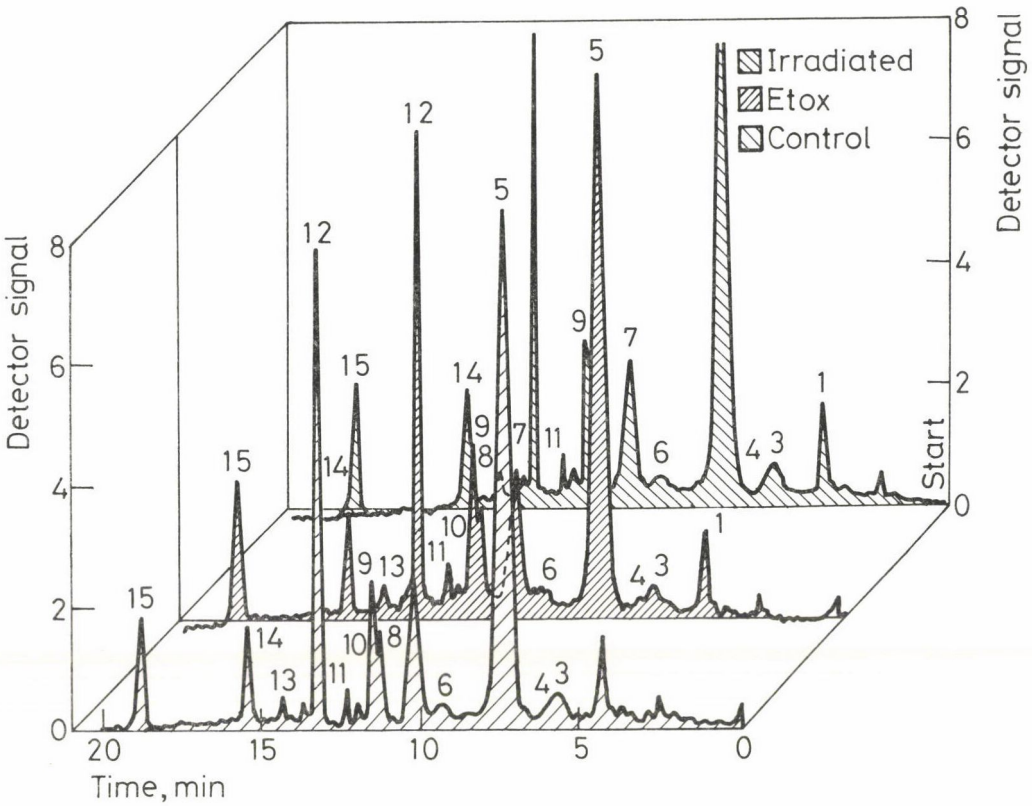

Fig. 1. Comparison of the carotenoid chromatograms of untreated, ethylene oxide treated and irradiated (5 kGy) ground paprika samples. For identification of the chromatographic peaks see the text

peak (not identified) (4); capsanthin (5); 9-cis-capsanthin (6); 13-cis-capsanthin (7); completely covering antheraxanthin of identical retention time; another non-identified component (8) partly overlapping the zeathin peak (9); 9-cis--zeaxanthin (10); 13-cis-zeaxanthin (11); internal standard cantaxanthin (12); beta-cryptocapsin (13); beta-cryptoxanthin (14) and finally beta-carotene (15). The chromatogram is a convincing proof of this method being an important advance in carotenoid analysis. Minute components may be determined by it for which earlier methods were not suitable.

It is also visible from the figure that neither of the treatments caused the appearance of new peaks or that the peaks shown in the chromatogram of the control sample are present in the chromatograms of treated samples, too. Thus, no qualitative change was caused by any of the treatments in the carotenoid composition of ground paprika.

2.2. Analysis of variance of the effect of treatments and storage time

In Figure 1 the triads of peaks belonging to the same carotenoids show great similarity in respect to height and area so seemingly big quantitative changes were not caused by the treatments. This can be proved definetely only by statistical evaluation of the data. Here the statistical results of only two relevant carotenoids will be demonstrated.

Data related to capsanthin were evaluated by analysis of variance as shown in Table 1.

Table 1

Capsanthin as percentage of total carotenoid contents as affected by treatment and storage time
Analysis of variance

Factor	Sum of squares	Degree of freedom	Mean of squares	F value
Total	192.37	44	4.37	
Storage time	149.68	4	37.42	44.02***
Treatments	10.93	2	5.47	6.44**
Interaction	6.26	8	0.78	0.92
Error	25.50	30	0.85	

*** Very highly significant at P = 99.9% probability level
** Highly significant at P = 95% probability level
$LSD_{5\%}$ = 1.54% capsanthin

Capsanthin as percentage of total carotenoid content[a]

Storage time (months)	Treatments		
	Control	Irradiation (5 kGy)	Ethylene-oxide[b]
0	52.3	55.7	54.4
2	51.5	52.7	52.2
3	51.0	51.0	51.8
4	48.7	49.0	48.9
5	49.7	51.1	51.4

[a] = values calculated from the chromatographic data
[b] = 6 h; 30–35 °C; ethylene oxide concentration 0.64 kg m^{-3}

As seen in the Table the percentage of capsanthin in total carotenoid content was very highly significantly (P = 99.9%) affected by storage time and it was highly significantly affected (P = 99%) by the treatments. The least significant difference (LSD 5%) was 1.54% capsanthin in absolute percentage. Comparing this result with the differences in the mean values as seen at the bottom of the Table the following can be concluded:

— Immediately after radiation treatment the capsanthin content of 55.7% was significantly higher than the capsanthin content of the untreated paprika, while ethylene oxide treatment did not cause a significant change.

— Until the 5th month of storage there was no difference observed in the samples treated by different methods. At this time, however, the proportion of capsanthin in the pigment of the ethylene oxide treated sample amounted to 51.4%, while that in the untreated sample was 49.7%.

Storage time affected the samples more than treatments as proved also by analysis of variance (F = 44.02). During the first two months of storage a significant reduction occurred in the proportion of capsanthin and this was independent of treatment. In the fourth month of storage the capsanthin percentage reached its minimum in all the samples whether treated or untreated (about 49%). In the 5th month the capsanthin percentage increased to 51.1% in the radiation treated sample and to 51.4% in the ethylene oxide treated one. This increase was significant while that of the control sample was not significant.

For beta-carotene the effect of storage time was significant at the P = 99.9% level as shown by analysis of variance (Table 2).

Table 2

Beta-carotene as percentage of total carotenoid content[a] *as affected by treatment and storage time*
Analysis of variance

Factor	Sum of squares	Degree of freedom	Mean of squares	F value
Total	15.81	44	0.36	
Storage time	10.89	4	2.72	28.11***
Treatments	0.82	2	0.41	4.22**
Interaction	1.19	8	0.15	1.55
Error	2.91	30	0.097	

*** Very highly significant at P = 99.9% probability level
** Highly significant at P = 95% probability level
$LSD_{5\%}$ = 0.52% beta-carotene

Beta-carotene as percentage of total carotenoid content[a]

Storage time (months	Treatments		
	Control	Irradiation (5 kGy)	Ethylene oxide[b]
0	6.8	6.2	7.0
2	7.2	6.9	6.8
3	7.7	7.2	7.7
4	7.9	8.0	8.1
5	7.1	6.8	6.8

[a] = values calculated from the chromatographic data
[b] = 6 h; 30–35 °C; ethylene oxide concentration 0.64 kg m^{-3}

The effect of radiation treatment and ethylene oxide treatment proved to be significant at the 95% probability level, only. The least significant difference in carotene percentage amounted to 0.52%.

At the beginning of storage the beta-carotene content of 6.2% of the pigment content of the irradiated sample was significantly lower than that of the control sample or of the ethylene oxide treated sample. After 2 month storage there was no difference between samples treated differently. In the third month the lowest percentage was found again in the radiation treated sample. During further storage the differences caused by different treatments were lower then the least significant difference.

In the fourth month the beta-carotene proportion in the pigment content of all the samples, whether treated or not, amounted to about 8%. In the 5th month a significant reduction occurred in all the samples.

3. Conclusions

In comparison to the control sample both methods of decontamination caused significant, however, from the practical point of view negligible changes in the carotenoid composition of ground paprika.

Storage time affected the proportions of individual carotenoid components more strongly than the treatments of sanitizing purpose.

The changes in the proportions of the scrutinized carotenoids during storage indicate that ground paprika is not a stabile system. On the basis of these data it can not be decided whether these proportional changes are the consequences of the different decomposition rates of carotenoids or that there exists the possibility of transformations of carotenoids during storage.

Stability of carotenoids upon both treatments was practically about the same. If we add to this the advantages of irradiation in respect of safety and microbiological efficacy we can conclude that irradiation is a promising new technology for the decontamination of spices.

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PACKAGING AND STORAGE EFFECTS ON THE QUALITY OF PLANT NUTS

A. SATTAR, M. WAHID, M. JAN, A. AHMAD and S. KHAN

Nuclear Institute for Food and Agriculture, Tarnab, Peshawar. Pakistan

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Plant nuts such as almond, groundnut, pinenut and walnut, were packed in clear, silver and black coloured polyethylene (PE) as well as in paper bags and amber glass and stored at ambient temperature (16–38 °C) for 6 months. One of the samples was irradiated with 0.50 kGy as well. Quality was measured in terms of peroxide value, instrumental colour values and sensory tests. Peroxidation of nuts increased with advanced storage, and the rate was higher in pinenut and walnut than almond and groundnut. Among the packages, amber glass protected the quality better than others. Radiation treatment showed no beneficial effect.

Keywords: packaging, keeping quality, irradiation, colour, peroxidation

Nuts are hard, one-stone fruits generally produced from a compound ovary. They probably originated in the hot, dry regions of Western Asia and were carried by early man to the areas around the Mediterranean and then elsewhere (WOODROOF, 1979). Dry fruits and nuts are a major source of income and foreign exchange in several countries and are utilized throughout the year (ITC, 1973). The spoilage of dry nuts as a result of insect infestation, microbial growth, and oxidative changes is a serious problem especially under hot-humid climates like that of Pakistan (ITC, 1973). Gamma irradiation has been tried for insect disinfestation in dry fruits and nuts (BROWER & TILTON, 1970, 1972; GUERRIERI, 1975). Effect of radiation doses and storage temperatures on the shelf life and quality of dry fruits and nuts has been established (SATTAR et al., 1989c; WAHID et al., 1987). Peroxidation of nut oils under dark and light conditions has also been studied (SATTAR et al., 1989b). Radiation effect on the insects infesting dry nuts has also been reported (WAHID et al., 1989). However, the influence of combination methods on biochemical and sensory quality of nuts has been studied less. The present work was, therefore, undertaken to investigate the effect of irradiation and coloured packaging materials, on the quality of dry nuts.

1. Materials and methods

Nuts such as almond *(Prunus amygdalus)*, groundnut *(Archis hypogaea)*, pinenut *(Alpinus pinea)* and walnut *(Juglans regia)* were obtained from a whole-sale dealer in a local market at Peshawar. They were thoroughly cleaned for insect infestation and visible quality. The cleaned, sorted samples 250 g of each nut in duplicate for each storage interval were packed in clear (not coloured), silver and black coloured polyethylene (PE) and paper bags of 20×15 cm as well as 500 cm^3 capacity amber coloured glass jars. The thickness of packaging materials such as clear (not coloured) PE, silver PE, black PE, paper bags and amber glass was 0.046, 0.045, 0.043, 0.050 and 2.03 mm, respectively. The silver and black coloured packages contained the respective dyestuffs in the PE. The light transmission of these packages was measured over a wavelength range of 200–1100 mm using UV-Vis recording Shimadzu (model UV-160) spectrophotometer. The clear (not coloured) PE transmitted 85.7%, silver PE 34.1%, black PE 5.1%, paper bag 1.7% and amber glass 14.0% of light (mainly between 500–1100 mm). A sample in each case, packed in clear (not coloured) PE was also irradiated with 0.5 kGy for comparison in a Cobalt–60 gamma radiator (Issledovated, USSR) having a dose rate of 8.75 kGy h^{-1} and max–min. ratio of 2.5. The samples were stored for 6 months at ambient room temperature (16–38 °C) and relative humidity 20–75% under normal light and dark conditions during day and night, respectively. The light intensity during the day was about 108 lx, as measured by means of General Electric type 214 light-meter.

Chemical analysis for peroxide value (POV) was carried out according to LEA (1952). In this method, a weighed (1 g) amount of extracted oil was mixed 1 g of KI and 20 cm^3 mixture of glacial acetic acid and chloroform (2 : 1), which was boiled for 1 min in a water-bath. The contents were transferred to a flask containing 20 cm^3 of 5% KI. Peroxide oxygen liberates iodine from KI and this was titrated with sodium thiosulphate using starch as an indicator. Colour Difference Computer of Dicom ND-504 DE (Nippon Denshoky Kogyo Co. Ltd. Japan) was used to obtain L, a and b colour values. The instrument was standardized with a white tile (X = 81.60, Y = 86.68, Z = 91.18) and a 3 cm diam. opening in the viewing port. Readings were taken from three different areas and the mean results were expressed as L, a and b values. The L value measures the lightness, a value indicates the redness and b values show the yellowness of the samples. Sensory evaluation was conducted by hedonic rating on a 9-point scale (LARMOND, 1977). At each storage interval, samples were evaluated once for overall acceptability based on the mean of colour, taste and flavour scores by a panel of 7 judges.

The data were analysed by analysis of variance and the LSD values at 1% level were measured for determination of significance. The coefficients of variation (CV) and standard deviations were also calculated (LITTLE & HILLS, 1972).

2. Results and discussion

Influence of different packaging materials on peroxidation is shown in Table 1. Obviously the rate of oxidation increased with advanced storage. Peroxidation was higher in pinenut and walnut than almond and groundnut. However, the extent of oxidation differed depending upon the packaging material. Initially, the POV of almond, groundnut, pinenut and walnut were 2.1, 2.0, 3.5 and 3.0 meq kg^{-1}, respectively. The values increased to range values of 9.4–15.0, 9.0–18.6, 13.0–28.0 and 12.0–30.0 meq kg^{-1} in almond, groundnut, pinenut and walnut, respectively, depending upon the package. Statistical treatment of the data indicated significant effect of packages and storage period on POV of nuts ($P < 0.01$) and the LSD values at 1% level have been provided for comparison. The results revealed highest peroxidation in the unpacked and unirradiated samples, least in the amber glass and intermediary in other cases. Statistically it was found that the performance of paper bags and silver PE as well as amber glass and black PE was identical. Among the packages, clear PE was found to be least effective in controlling oxidation. There was slightly higher development of POV in the irradiated samples which was perhaps due to radiation induced acceleration of oxidation. Since the samples were kept under normal day light and dark conditions higher peroxidation in clear PE is understandable. Although statistically similar, slightly better performance of amber glass (transmission 14%) than that of black PE (transmission 5.1%) is rather interesting. This might be due to the heat-absorption effect of black PE. Although the effect of packaging on dry nuts has not been reported, yet superiority of amber glass over other packages has been shown in relation to milk and juices (SATTAR et al., 1983; SATTAR et al., 1989a). It was inferred that the better protection property of amber glass is due primarily to its ability to complete absorption of the damaging wavelengths of the light spectrum (200–500 nm). Paper bag, although reduced much of the incident light (1.7%, but it is considered more pervious to gases i.e. oxygen which increase oxidation. JAN and co-workers (1988) found that PE was not a satisfactory material for walnut and recommended packaging under nitrogen or in tin cans and subsequent storage at 20 °C. Similar findings were reported by WAHID and co-workers (1987) for dry fruits. Insects which damage dry fruits and nuts during storage have been shown by SATTAR and co-workers (1989c) and WAHID and co-workers (1987). Comparative rate of oxidation and fatty acid composition in nuts has also been reported earlier

(SATTAR et al., 1989b) where it was shown that differential oxidation of almond, groundnut, pinenut and walnut was related to their fatty acid composition.

Instrumental colour measurements of plant nuts showed wide differences during 6 months storage (Table 2). Colour Difference Computer L values (lightness of colour) were higher at 6 months than at the beginning in all the stored nuts. Higher L values on storage indicated an increase in redness in relation to freshly stored samples. All plant nuts also had higher b values indicating an increase in the yellowness of the sample. The data also revealed that unpacked and paper and clear PE packed samples showed little difference in L, a and b values but those kept in black colour PE or amber glass exhibited higher variations than the other treatments indicating protection of original colour of the dry nuts to a certain extent. Statistical treatment of the data showed highly significant influence of storage and packaging ($P < 0.01$) on the colour values of these nuts.

Sensory evaluations were conducted to see if the consumer is capable to perceive colour and other differences in stored nuts. The influence of different packaging and storage on the organoleptic quality of these nuts was therefore, monitored and the results on overall acceptability for colour, texture and flavour are presented in Table 3. The range rating values decreased from 7.9–8.2 to 4.0–5.1, 7.0–7.4 to 4.0–5.1, 7.0–7.1 to 3.0–4.8 and 7.1–8.2 to 3.0–4.8 in almond, groundnut, pinenut and walnut, respectively, during 2–6 months storage depending on the packaging. Statistical analysis of organoleptic scores indicated significant effect of packaging and storage period ($P < 0.01$). It was observed that the colour and appearance of the samples were quite attractive at the beginning of the experiment, which changed from light to dark shades during storage at room temperature. This shows an association with the values obtained with Colour Difference Computer. Marked damaging effect of storage was found on the overall acceptability scores which exhibited decreasing pattern especially with extended storage. Packaging of the nuts in black PE pouches or amber glass resulted in better product than other tested packages. Radiation treatment with 0.50 kGy did not show any improvement because packaging was done in clear PE. FARKAS and co-workers (1974) and SARVACOS and MARCIS (1963) also did not find any significant effect of irradiation treatment (5.0–5.4 kGy) on the colour or flavour of dry fruits. KOVÁCS and co-workers (1977) compared the sensory and instrumental colour difference values and showed good correlation.

A similar trend was observed in the present work. JAN and co-workers (1988) found no statistically significant difference between packed unirradiated and irradiated (0.5 and 1.0 kGy) samples of walnuts. As a result of these studies it was concluded that packaging of plant nuts in amber coloured glass jars would greatly help in maintaining the quality of these food materials of high fat content during storage.

Table 1

Effect of packaging materials on the peroxide value (meq kg^{-1}) of dry nuts

Packaging material	Duration of storage (month)												Mean value	CV
	2	4	6	2	4	6	2	4	6	2	4	6		
	Almond			Groundnut			Pinenut			Walnut				
Unpacked-unirradiated	3.5	5.5	15.0	3.8	5.6	18.6	6.0	6.7	28.0	4.8	8.3	30.0	11.3	83.5
Clear PE + radiated	3.0	4.9	13.5	3.1	4.4	14.0	5.6	6.5	25.2	4.5	5.9	26.2	9.7	85.0
Clear PE	2.9	4.5	13.4	2.8	3.9	12.5	5.7	6.0	25.0	3.8	4.9	26.0	9.3	89.6
Silver PE	2.6	4.6	11.0	2.7	3.4	11.2	5.0	6.0	20.6	3.5	4.6	20.5	8.6	8.25
Black PE	2.5	4.2	9.7	2.4	3.8	9.2	4.5	5.2	14.9	3.4	3.9	13.1	8.0	81.9
Paper bags	2.8	4.8	11.2	2.9	3.6	12.5	5.7	6.0	23.7	3.9	5.0	20.5	6.4	66.3
Amber glass	2.2	3.7	9.4	2.3	3.6	9.0	4.0	5.0	13.0	3.4	4.0	12.0	6.0	64.0
Standard deviation of mean value	±0.20	±0.35	±0.89	±0.22	±0.31	±0.99	±0.33	±0.45	±2.1	±0.21	±0.31	±2.6		
Mean values	2.6	4.3	11.0	2.7	3.9	11.1	5.2	6.0	19.1	3.7	4.7	18.6		
CV	10.9	12.5	16.7	9.3	10.1	19.1	7.7	4.5	26.4	11.0	14.0	33.8		
LSD-1% level Storage time	0.411			0.605			1.119			0.604				
Packaging	0.485			0.235			0.389			0.509				

Initial POV: almond 2.1, groundnut 2.0, pinenut 3.5 and walnut 3.0 meq kg^{-1}
Storage temperature: 16–38 °C
CV: coefficient of variation (sample standard deviation expressed as a percentage of sample mean)

Table 2

Effect of irradiation and packaging on instrumental colour values of dry nuts

Dry nuts	Almond			Groundnut			Pinenut			Walnut		
	L	*a*	*b*	*L*	*a*	*b*	*L*	*a*	*b*	*L*	*a*	*b*
Unpackaged-unirradiated	30.7	21.7	8.2	46.9	22.3	9.8	52.0	19.0	15.4	35.8	15.5	3.3
Clear PE + irradiated	30.6	21.1	7.9	46.5	22.4	9.6	55.2	19.7	17.3	38.2	18.3	3.0
Clear PE	30.8	21.5	8.0	46.8	22.2	9.7	55.3	19.2	15.8	36.0	16.2	3.4
Silver PE	31.4	21.4	7.8	49.8	20.8	6.6	60.4	16.7	12.6	40.8	13.7	2.1
Black PE	32.8	20.6	7.5	50.6	20.2	6.7	62.8	15.9	12.2	41.5	12.9	2.0
Paper bags	30.5	21.6	8.0	46.4	22.4	9.7	55.8	19.4	15.6	36.1	16.0	3.5
Amber glass	33.4	20.2	7.6	50.4	20.0	6.7	62.9	15.6	12.0	41.6	12.7	2.0
Standard deviation of mean value	±2.5	±1.50	±0.80	±3.50	±2.00	±0.92	±5.50	±3.00	±2.00	±3.63	±2.55	±0.26
Mean values	31.5	2.12	7.9	48.2	21.5	8.4	57.8	17.9	14.4	38.6	15.0	2.8
CV	3.7	2.7	3.1	4.1	5.1	19.3	7.4	10.0	14.6	7.0	13.6	24.0
LSD-1% level												
Colour value	2.723			1.120			1.119			1.255		
Packaging	0.439			0.615			0.455			0.379		

Initial values: almond *L:* 36.6, *a:* 16.4, *b:* 6.7; groundnut *L:* 54.9, *a:* 28.4, *b:* 6.0; pinenut *L:* 64.5, *a:* 13.2, *b:* 10.5; walnut *L:* 44.4, *a:* 10.6, *b:* 1.6

Storage temperature of six months: 16–38 °C

CV: coefficient of variation (sample standard deviation expressed as percent of sample mean)

Table 3

Effect of packaging material on sensory quality of dry nuts

Packaging material	Duration ofstorage (month)												Mean value	CV
	2	4	6	2	4	6	2	4	6	2	4	6		
	Almond			Groundnut			Pinenut			Walnut				
Unpackaged-unirradiated	8.0	7.0	4.0	7.0	6.0	4.5	7.0	5.5	3.0	7.1	5.2	3.0	5.6	30.2
Clear PE + irradiated	8.0	7.2	4.0	7.0	6.3	4.9	7.0	5.6	3.5	7.1	5.3	3.7	5.7	27.9
Clear PE	7.9	7.0	4.0	7.1	6.0	4.9	7.0	5.6	3.9	7.1	5.0	3.2	5.7	27.0
Silver PE	8.1	7.3	4.7	7.2	6.9	4.3	7.1	6.0	4.6	7.9	5.7	4.0	5.8	27.3
Black PE	8.2	7.4	5.1	7.4	7.4	4.1	7.1	6.7	4.8	8.0	6.0	4.5	6.2	22.6
Paper bags	8.0	7.0	4.6	7.0	6.2	4.0	7.0	5.4	4.0	7.5	5.0	3.1	6.5	20.7
Amber glass	8.2	7.4	5.1	7.4	7.3	5.7	7.1	6.7	4.8	8.2	6.4	4.8	6.5	19.6
Standard deviation of mean values	±0.25	±0.25	±0.40	±0.23	±0.33	±0.22	±0.34	±0.28	±0.22	±0.22	±0.30	±0.21		
Mean values	8.1	7.2	4.8	7.2	6.7	4.8	7.1	6.1	4.3	7.7	5.7	3.9		
CV	1.5	2.6	14.6	3.2	9.5	6.0	1.1	9.7	16.8	6.0	10.6	20.3		
LSD-1% level Storage time	1.034			1.249			0.616			0.503				
Packaging	0.458			0.498			0.778			0.479				

Scores are the mean of 7 judgements for colour, taste and flavour
Scoring scale: 1–9 (1 extremely disliked, 9 extremely liked)
Storage temperature: 16–38 °C
CV: coefficient of variation (sample standard deviation expressed as percent of sample mean)

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EFFECTS OF ADDITIVES ON COLOUR STABILITY OF SLICED BOLOGNA TYPE SAUSAGE MADE OF PORK

Gy. Urbányi,[a] J. Farkas,[a] V. Mihályi,[b] K. Incze,[b] K. Horti[a] and K. Huszár[a]

[a]Institute of Preservation and Livestock Products Technology, University of Horticulture and Food Industry, H-1118 Budapest, Ménesi út 43–45. Hungary
[b]National Meat Research Institute, H-1097 Budapest, Gubacsi út 6/b. Hungary

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The nitroso-heme pigment lending a desirable colour to cured meat products is very sensitive to light and oxygen and the interplay of these two factors is responsible for the discolouration of the surface of sliced meat products. The development and stability of colour may be improved by additives. In the course of these experiments the effect exerted by some such additives was studied.

The pH and residual nitrite content in final products made of pork, was determined. Colour stability (objective colour measurement, sensory test, determination of the pigment content) was established directly after manufacture and after 1 week cold storage. The sliced product was exposed to light for four hours during which a sample was tested every hour.

The comparison of the results of sensory tests and instrumental colour measurement has shown that the Momcolor instrument is more sensitive to differences in colour then the sensory panel. At the same time in the case of substantial colour differences the agreement between the observed and instrumentally measured values, was close.

Keywords: colour stability, colour of sausages

Of the additives applied to stabilize the colour, "Tari", a product available on the market, and ascorbic acid increased substantially the redness of the meat product while the mixture was less effective. Glucose or Na-glutamate had a definitely detrimental effect on the red colour. The additives used to improve colour did not exert a stabilizing effect in the sense that they did not slow down the break down of the component responsible for the red colour sensation during exposure to light or oxygen.

During the one week cold storage, colour development continued and the nitroso pigment continued to be formed.

It was found in the experiments that the factors improving the red colour but not improving colour stability (i.e., they did not slow down the transformation of the nitroso pigment), with the help of the factors increasing initial nitroso pigment formation it is possible to keep the colour of the slices acceptable for 1–2 h longer.

The nitroso-heme pigment, lending a desirable colour to cured meat products is sensitive to light and oxygen. The interplay of these two factors

cause fading of the surface of the sliced product, and its brownish, greyish discolouration (ERDMAN & WATTS, 1957; TARLAGDIS, 1962; FOX, 1966; BARTON, 1967; ACTON et al., 1986; YEN et al., 1988). The process of discolouration probably consists in two phases. In the first phase the nitroso group, induced by light and catalysed by the presence of oxygen, dissociates from the pigment (TARLAGDIS, 1962). This phase is followed by the oxidation of the nitroso group (TARLAGDIS, 1962; FOX, 1966). Light serves as source of energy for oxidation. Some of the publications indicate the possibility that the pyrrole structure of heme may be oxidized, too (ERDMAN & WATTS, 1957; TARLAGDIS, 1962).

In the course of production it is possible to improve colour development by the use of additives. OKAYAMA and co-workers (1982) investigated the effect of sarcoplasm fractions of low molecular weight, glutathione (GSH), inosine monophosphate (IMP) and adenosine triphosphate (ATP) upon colour development in aqueous myoglobin solution, that was used as a model substance, and in sausage made of pork. GSH was found to promote colour development and the break down of nitrite. This effect can be increased by the addition of ATP, IMP and ribose.

OKAYAMA and co-workers (1989) studied the effect of ribose, xylose and arabinose upon colour formation in myoglobin model solutions under anaerobic conditions. Their results have shown ribose to improve strikingly colour formation. Similar effects were shown by the application of xylose and arabinose. The effect increases with increasing pH.

The aim of these investigations was to determine the degree to which stability of colour in slices of a cooked, cured meat product exposed to light is affected by addition of substances capable of stabilizing colour. Samples exposed to light and oxygen for periods between 0 to 4 h were evaluated by sensory tests and instrumental measurements.

1. Materials and methods

1.1. Materials

"Normal" pork was used for the samples. The product was a Bologna type sausage without seasoning (curing mixture: 1.8% nitrited salt containing 0.5% Na nitrite). The different additives were mixed into the meat mass and allowed to stand for 2 h at 15–20 °C. Then the mass was filled into synthetic casing (NALOTOP) (4 cm diameter and 20 cm length) and clipsed. That was impermeable to water. The sausages were heated at 72 °C for 40 min. The sausages were not exposed to smoking.

The following additives were used (serial numbers serve to identify the samples):

1. No additive (control),
2. ascorbic acid, p.a., Reanal (200 mg per kg),
3. commercial mixture (1000 mg per kg),
4. ascorbic acid–glucose–natrium-L-glutamate mixture (1000 mg per kg),
5. glucose, p., Reanal (450 mg per kg),
6. natrium-L-glutamate, p., Reanal (350 mg per kg).

Half of the samples were studied on the first two days after production while the second half were kept in a household refrigerator (at ca 5 °C) for one week, then taken out and sliced and examined.

1.2. Methods

1.2.1. *Measurement of pH* with a Radelkis digital pH meter (Type: OP-211(1), Hungary.

1.2.2. *Determination of the residual nitrite content* by Griess—Ilosvay reagents in accordance with the HUNGARIAN STANDARD (1981) method.

1.2.3. *To test colour stability and visual ranking*, slices 10 mm thick were used. To determine pigment content, slices 1 mm thick were used, all slices were cut with an electric slicing machine. To lessen drying of the slices they were kept in empty petri dishes at room temperature. Illumination by daylight in the laboratory was complemented by fluorescent lamp illumination. The six 40 W fluorescent lamps were fixed at 2 m height.

1.2.3.1. *Objective measurement of colour* — The colour of meat and meat products can be measured with tri-stimulus colour sensation measuring instruments (KLETTNER & STREBING, 1980). In these experiments the MOMCOLOR-D (Hungarian Optical Works) tri-stimulus colour sensation measuring instrument was used by reflection method. The colour of the slices was established immediately after slicing and after 1, 2 and 4 h exposure to air and light. Measurements were made on 3 slices at 3 places on each sample, giving nine parallel measurements. During the measurements, to avoid disturbing reflections, the slices were under a black cover. The samples were related to the official white enamel etalon of the National Bureau of Measurements (No. 80-26-00). This standard has the following colour characteristics:

$$X_1 = 64.90 \; X_2 = 15.82 \; Y = 82.91 \; Z = 95.14.$$

The geometry of the measuring head was $0^0/45^0$ at 2^0 angle of vision and C.I.E. C illumination. The diameter of the diaphragm was 10 mm. The results of measurements were used to calculate the colour sensation characters in the CIELAB system.

1.2.3.2. *Sensory colour test* — Parallel to the instrumental measurement, a panel of five judges carried out a colour ranking test. The task of the panel

members was to rank the slices according to their surface colour. The rank numbers were statistically evaluated according to the method of BASKER (1988).

1.2.3.3. *Analysis of pigment content* — The pigment content of the samples and its changes were established by extracting the meat pigment and measuring absorbance with a spectrophotometry. The method was essentially that of HORNSEY (1956) as modified by GANTNER (1959/60).

Slices of only 1 mm thickness were used in order to lessen the effect that internal pigments would have on the measurements.

To determine the total pigment content 2.5–3 g slices were pulped in a four-fold quantity of 80% acetone acidified with HCl (acetone–water–conc. HCl, 80 : 18 : 2) and the test-tubes containing the pulp were covered with parafilm and allowed to stand for 30 min in the dark. After extraction, the solutions were filtered through folded filter paper and their absorption spectrum against the pure solvent was established on a Beckman DU64 spectrophotometer.

The nitroso pigment was extracted by the same method, however the solvent was not acidified with HCl. To prevent the breakdown of the light-sensitive nitroso pigment the solution was acidified with 1 cm^3 HCl directly after filtration. Thus the extracted nitroso pigment was converted to hydrochloric hematin, which is not sensitive to light.

2. Results and conclusions

2.1. pH of the products

The results of pH measurement on the day following production and one week after production in cold storage are given in Table 1.

Table 1

The residual nitrite content after production and pH of the products upon testing

Sample[a]	Residual nitrite (mg $NaNO_2$ kg^{-1})	pH	
		The day after processing	After one week cold storage[b]
1	39.0	6.38	6.49
2	25.0	6.48	6.49
3	26.2	6.47	6.52
4	26.4	6.47	6.53
5	35.0	6.50	6.56
6	37.0	6.49	6.55

[a] 1: Control, 2: ascorbic acid (200 mg per kg), 3: commercial mixture (1000 mg per kg), 4: ascorbic acid–glucose–Na-glutamate mixture (1000 mg per kg), 5: glucose (450 mg per kg), 6: Na-glutamate (350 mg per kg)

[b] 5 °C

As seen in the Table the pH of the samples prepared with different additives did not differ. The pH of the control somple was somewhat lower than that of the other samples when examined the day after processing. All samples increased somewhat in pH during the one-week storage period.

2.2. Residual nitrite content of the products

The residual nitrite contents are also shown in Table 1. The residual nitrite content in the samples containing glucose and Na-glutamate (No. 5, 6) did not differ appreciably from that of the control sample (No. 1) while the samples with added ascorbic acid, commercial mixture or a mixture of additives (No. 2, 3 and 4), respectively, had a substantially lower nitrite content but not differing from one another. This shows that the reaction causing red colour takes place more completely in samples 2–4 than in the control sample.

2.3. Colour measurements

The results of colour measurements carried out in the samples, both fresh and stored, prepared with five different additives are summarized in Tables 2, 3, 4 and 5.

2.3.1. Measurements carried out on the day following production

2.3.1.1. Effect of additives upon colour — The measurements carried out on the day following production show that the best colour by far was achieved with the commercial mixture (No. 3). This sample had the reddest colour with the least yellow in it and was the darkest. The red character of this sample was significantly better at the 95% level than of those prepared with ascorbic acid (No. 2) or the mixture of additives (No. 4). The yellow character was also significantly better at the 95% level than that of all the other samples, except the one made with ascorbic acid. The colour of the samples prepared with glucose and Na-glutamate additives (No. 5 and 6) was significantly worse than that of all other samples.

After 1 h illumination the samples prepared with the commercial mixture became second as regards red character. The product prepared with ascorbic acid appeared redder, however, the difference was not significant.

As redness diminished in all samples, their redness values became less different.

After 2 h illumination the sample containing commercial mixture (No. 3) was again first as regards red character. At the same time the colour of the other two samples (No. 2 and 4) considered good so far approached the colour of the control sample. The difference between the colours of the three samples is not significant.

Table 2

CIELAB colour characteristics of samples prepared with different additives (t = 0 h)

Sample[a]		a*	b*	L*	C*	h°
	The day after processing					
1	$\bar{x}$	5.05^{ad}	10.23^{a}	73.18^{abcde}	11.42	63.82
	±s	0.29	0.16	0.11	0.25	1.07
2	$\bar{x}$	7.05^{bc}	8.81^{bcd}	73.27^{abcde}	11.29	51.36
	±s	0.19	0.11	0.14	0.09	1.00
3	$\bar{x}$	7.17^{bcd}	8.28^{bc}	72.62^{abc}	10.98	49.19
	±s	0.31	0.17	0.13	0.08	1.78
4	$\bar{x}$	5.80^{acd}	9.23^{bd}	73.51^{abde}	10.92	57.85
	±s	0.27	0.15	0.11	0.03	1.63
5	$\bar{x}$	2.40^{ef}	11.59^{ef}	73.47^{abdef}	11.84	78.33
	±s	0.11	0.07	0.18	0.07	0.56
6	$\bar{x}$	2.49^{ef}	11.63^{ef}	74.22^{ef}	11.90	77.90
	±s	0.11	0.04	0.16	0.04	0.56
	After one week cold storage at 5 °C					
1	$\bar{x}$	6.19^{ad}	9.51^{a}	73.30^{abcde}	11.36	56.99
	±s	0.19	0.08	0.20	0.06	1.01
2	$\bar{x}$	8.15^{bc}	8.68^{bd}	73.64^{abcde}	11.91	46.83
	±s	0.13	0.04	0.13	0.09	0.50
3	$\bar{x}$	7.85^{bcd}	8.14^{c}	72.87^{abce}	11.31	46.08
	±s	0.19	0.06	0.22	0.15	0.71
4	$\bar{x}$	6.98^{acd}	8.70^{bd}	73.83^{abdef}	11.16	51.32
	±s	0.21	0.05	0.13	0.10	0.96
5	$\bar{x}$	3.24^{ef}	11.32^{ef}	73.73^{abcdef}	11.78	74.05
	±s	0.13	0.04	.016	0.05	0.64
6	$\bar{x}$	3.17^{ef}	11.39^{ef}	74.20^{def}	11.83	74.44
	±s	0.14	0.03	0.06	0.03	0.67

Letters mark significant differences at 95% probability level. For a given column, figures marked with the same letter do not differ significantly.

$\bar{x}$ = average

±s = standard deviation

For sample see Table 1.

During the fourth hour of illumination the colours of the samples became even more alike. The red character of the sample containing commercial mixture was further reduced. After 4 h the control sample had the best colour but the difference was not significant. In relation to the yellow character, the same sample was the best (of the lowest b* value), too. Lightness of colour did not differ significantly between the samples. The samples prepared with glucose and Na-glutamate (No. 5 and 6) showed the poorest colour all through. Except

Table 3

CIELAB colour characteristics of Bologna samples prepared with additives (t = 1 h)

Sample		a*	b*	L*	C*	h°
		The day after processing				
1	$\bar{x}$	3.88^{acd}	12.24^{abef}	73.16^{abcde}	12.84	72.44
	$\pm s$	0.16	0.07	0.22	0.11	0.65
2	$\bar{x}$	4.75^{bc}	12.00^{ab}	73.35^{abcdef}	12.90	68.42
	$\pm s$	0.09	0.04	0.22	0.03	0.40
3	$\bar{x}$	4.53^{abcd}	11.54^{cd}	72.53^{abcd}	12.41	68.58
	$\pm s$	0.22	0.11	0.21	0.12	0.98
4	$\bar{x}$	4.06^{acd}	11.87^{cd}	73.45^{abcdef}	12.55	71.13
	$\pm s$	0.16	0.05	0.20	0.05	0.75
5	$\bar{x}$	2.23^{ef}	12.47^{aef}	73.80^{abdef}	12.67	79.85
	$\pm s$	0.05	0.03	0.15	0.03	0.23
6	$\bar{x}$	2.28^{ef}	12.56^{aef}	74.11^{bdef}	12.77	79.71
	$\pm s$	0.03	0.07	0.13	0.07	0.15
		After 1 week cold storage at 5 °C				
1	$\bar{x}$	5.07^{abcd}	11.89^{a}	73.23^{abcdef}	12.93	66.94
	$\pm s$	0.20	0.05	0.12	0.11	0.80
2	$\bar{x}$	5.79^{abcd}	11.51^{bd}	73.19^{abcdef}	12.89	63.30
	$\pm s$	0.13	0.04	0.13	0.07	0.50
3	$\bar{x}$	5.67^{abcd}	11.00^{e}	72.50^{abce}	12.38	62.74
	$\pm s$	0.12	0.06	0.22	0.09	0.48
4	$\bar{x}$	5.21^{abcd}	11.30^{bd}	73.47^{abdef}	12.45	65.27
	$\pm s$	0.18	0.05	0.07	0.09	0.78
5	$\bar{x}$	2.75^{ef}	12.19^{ef}	73.29^{abcdef}	12.50	77.32
	$\pm s$	0.18	0.03	0.11	0.05	0.78
6	$\bar{x}$	2.55^{ef}	12.31^{ef}	73.82^{abdef}	12.57	78.32
	$\pm s$	0.13	0.03	0.12	0.04	0.58

For samples see Table 1

in the measurement after 4 h illumination in red character they differed significantly from the other samples.

2.3.1.2. Change in colour on the surface as a function of time — Both, the red and yellow character of the control sample (No. 1) suffered the most substantial change in the first hour. The red character diminished while the yellow character increased. There was no change in the lightness of colour. During further illumination the trend with respect to red and yellow colours was similar, however, of lesser degree (Fig. 2).

The ascorbic acid containing sample (No. 2) initially of better red colour than No. 3, suffered a similar trend, however, to a greater extent. During the

Table 4

CIELAB colour characters of Bologna samples prepared with additives (t = 2 h)

Sample		a*	b*	L*	C*	h°
		The day after processing				
1	$\bar{x}$	3.71^{abcd}	12.89^{abcf}	73.23^{abcdef}	13.42	73.98
	$\pm s$	0.21	0.03	0.14	0.06	0.88
2	$\bar{x}$	3.80^{abc}	12.88^{abdef}	73.36^{abdef}	13.43	73.58
	$\pm s$	0.08	0.08	0.16	0.08	0.35
3	$\bar{x}$	4.17^{abcd}	12.35^{cdef}	72.59^{ace}	13.08	71.54
	$\pm s$	0.57	0.08	0.13	0.28	2.13
4	$\bar{x}$	3.17^{acd}	12.56^{bcdef}	73.36^{abdef}	12.96	75.84
	$\pm s$	0.09	0.06	0.11	0.06	0.38
5	$\bar{x}$	2.26^{ef}	12.70^{abcdef}	73.21^{abcdef}	12.90	79.93
	$\pm s$	0.13	0.06	0.17	0.07	0.54
6	$\bar{x}$	2.21^{ef}	12.72^{abcdef}	73.66^{abdef}	12.91	80.15
	$\pm s$	0.11	0.08	0.20	0.09	0.46
		After 1 week cold storage at 5 °C				
1	$\bar{x}$	4.24^{abcd}	12.62^{abe}	73.11^{abde}	13.32	71.46
	$\pm s$	0.22	0.01	0.10	0.07	0.91
2	$\bar{x}$	4.80^{abcd}	12.42^{abdef}	73.30^{abdef}	13.32	68.92
	$\pm s$	0.22	0.08	0.17	0.15	0.76
3	$\bar{x}$	4.35^{abcd}	11.94^{cd}	72.30^{ce}	12.71	70.01
	$\pm s$	0.12	0.05	0.20	0.07	0.49
4	$\bar{x}$	3.93^{abcd}	12.11^{bcd}	73.26^{abde}	12.73	72.03
	$\pm s$	0.14	0.04	0.11	0.07	0.56
5	$\bar{x}$	2.48^{ef}	12.61^{abef}	73.11^{abcdef}	12.86	78.90
	$\pm s$	0.10	0.02	0.13	0.01	0.45
6	$\bar{x}$	2.45^{ef}	12.59^{bef}	73.73^{bf}	12.83	79.00
	$\pm s$	0.14	0.03	0.04	0.04	0.62

For samples see Table 1

fourth hour of illumination the colour of the two samples (No. 2 and 3) was practically identical.

The sample made with additive commercial mixture (No. 3, Fig. 2) had the best colour when freshly sliced. After 1 h the colour showed a relatively significant change, while in the second hour hardly any.

The colour points of sample No. 4 deteriorated evenly as seen in Fig. 2. This became apparent in the reduction of the red character and the increase of the yellow character.

The samples (No. 5 and 6) made with glucose and glutamate, respectively (Fig. 2), initially of poor colour, hardly changed as regards to red character. Deterioration of colour was apparent only in the increase of the yellow charac-

Table 5

CIELAB colour characters of Bologna samples prepared with additives (t = 4 h)

Sample		a*	b*	L*	C*	h°
		The day after processing				
1	$\bar{x}$	$3.25^{abcéf}$	13.01^{abcdef}	72.98^{abcdef}	13.43	76.04
	±s	0.28	0.10	0.28	0.17	1.06
2	$\bar{x}$	3.20^{ab}	13.31^{ab}	72.90^{abcdef}	13.70	76.50
	±s	0.15	0.03	0.15	0.04	0.63
3	$\bar{x}$	2.92^{ac}	12.74^{acdef}	72.26^{abcdef}	13.07	77.08
	±s	0.06	0.04	0.24	0.04	0.26
4	$\bar{x}$	2.61^{def}	12.88^{acdef}	73.21^{abcdef}	13.15	78.54
	±s	0.15	0.08	0.24	0.09	0.63
5	$\bar{x}$	2.36^{adef}	12.88^{acdef}	72.80^{abcdef}	13.10	79.61
	±s	0.15	0.05	0.20	0.05	0.66
6	$\bar{x}$	2.25^{adef}	12.99^{adef}	73.33^{abcdef}	13.19	80.16
	±s	0.12	0.04	0.19	0.04	0.53
		After 1 week cold storage at 5 °C				
1	$\bar{x}$	3.73^{abcd}	13.06^{ab}	73.07^{abdef}	13.59	74.09
	±s	0.20	0.02	0.17	0.08	0.80
2	$\bar{x}$	3.60^{abc}	13.14^{ab}	$73i77^{abdef}$	13.62	74.70
	±s	0.10	0.06	0.17	0.07	0.40
3	$\bar{x}$	3.14^{abcd}	12.71^{cdef}	72.43^{c}	13.10	76.12
	±s	0.11	0.08	0.07	0.10	0.39
4	$\bar{x}$	2.96^{acd}	12.75^{cdef}	73.06^{abdef}	13.10	76.94
	±s	0.10	0.07	0.16	0.07	0.42
5	$\bar{x}$	2.35^{ef}	12.82^{cdef}	72.93^{abdef}	13.03	79.64
	±s	0.11	0.06	0.11	0.06	0.48
6	$\bar{x}$	2.27^{ef}	12.85^{cdef}	73.49^{abdef}	13.05	79.99
	±s	0.10	0.04	0.12	0.04	0.44

For samples see Table 1

ter. It is worth mentioning that the red character of these samples was lower on the freshly cut surface than on the surface of the other samples after 4 h exposure.

2.3.2. Tests carried out after one week cold storage. Generally it can be said that the red character of the samples increased while their yellow character decreased during the one week storage (Table 2).

2.3.2.1. The effect of additives upon colour — Measured on the freshly cut surface (Fig. 3) of the sample kept for one week in cold storage as regards the red character the ascorbic acid containing sample and as regards the yellow character the "TARI" containing sample proved to be the best. However, the difference is significant only in the yellow character.

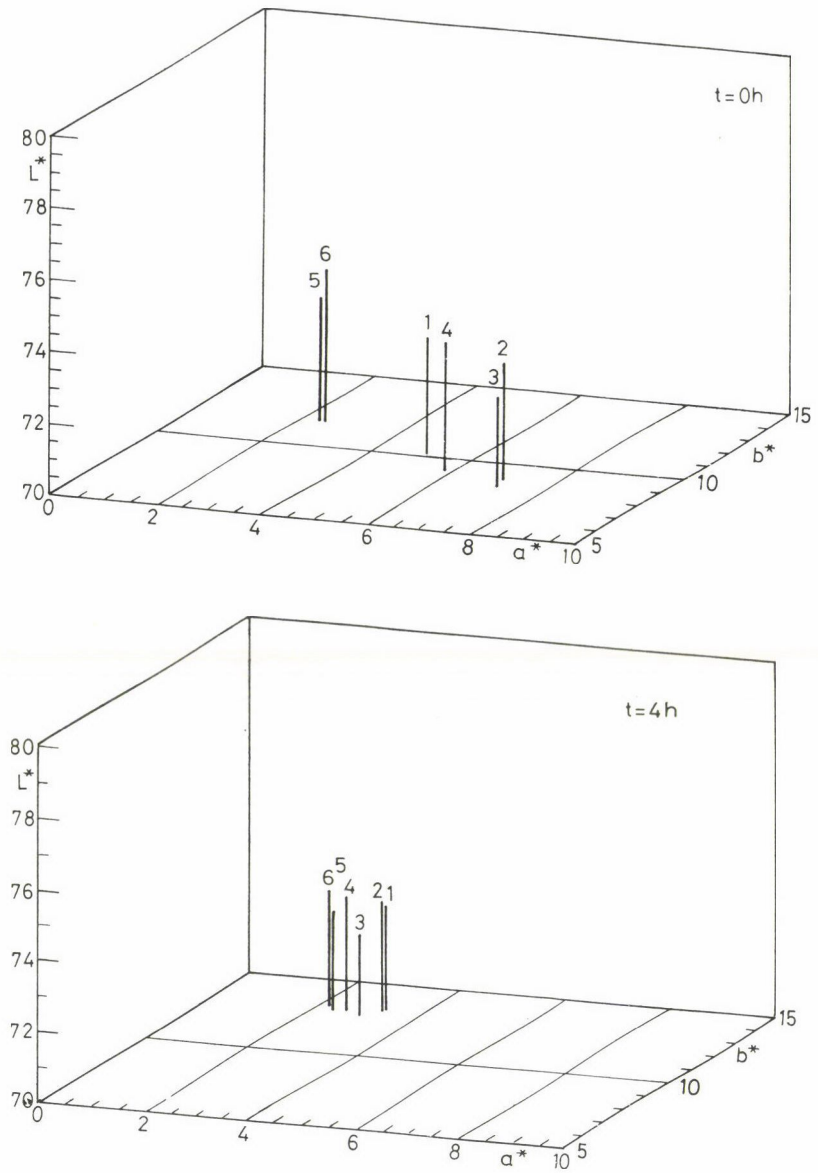


Fig. 1. Colour points of samples freshly sliced (t = 0 h) and illuminated during 4 hours (t = 4 h) in the CIELAB colour stimulus space. 1: Control; 2: ascorbic acid; 3: commercial mixture; 4: mixture; 5: glucose; 6: Na-glutamate

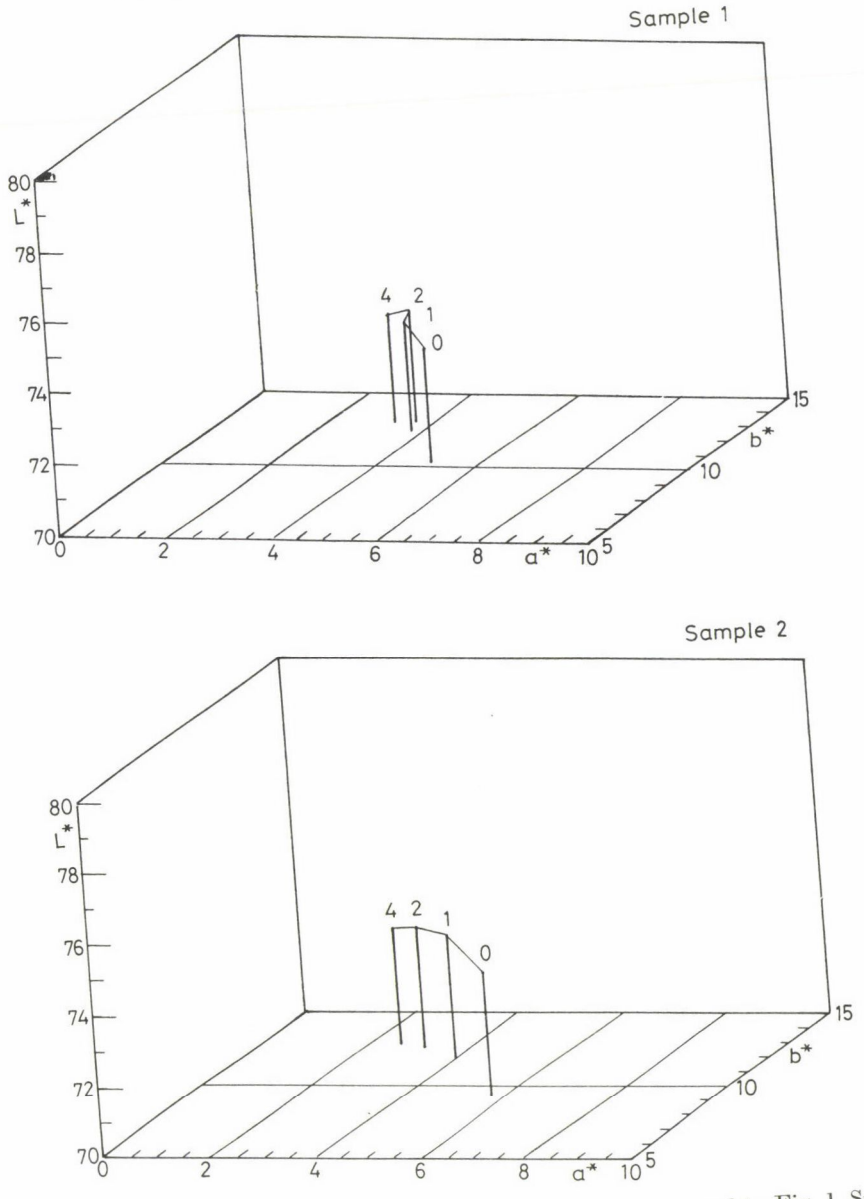


Fig. 2. Colour points versus illumination time. For samples see legend to Fig. 1. Symbols: 0: freshly cut surface (t = 0); 1: illumination for one hour (t = 1); 2: illuminated for 2 h (t = 2); 4: illuminated for 4 h (t = 4)

(Figures of samples No. 3—6 are presented on pages 142—143)

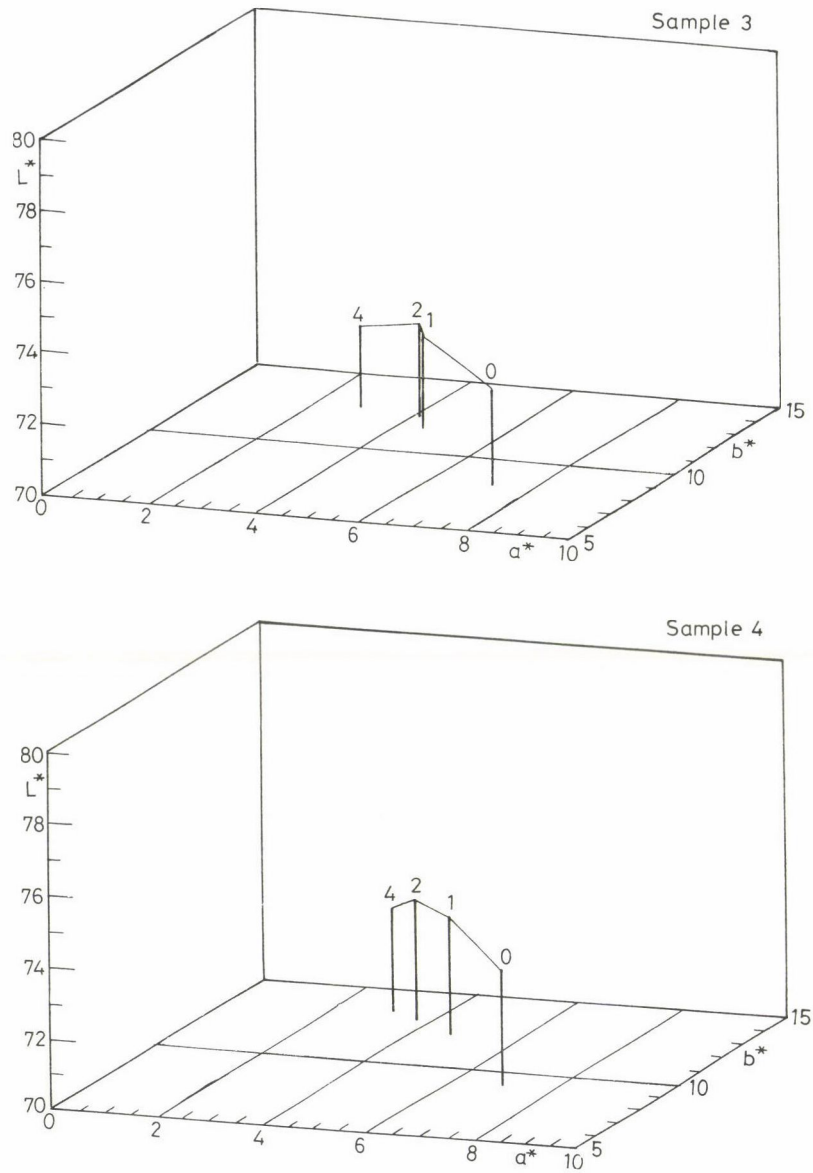


Fig. 2. continued

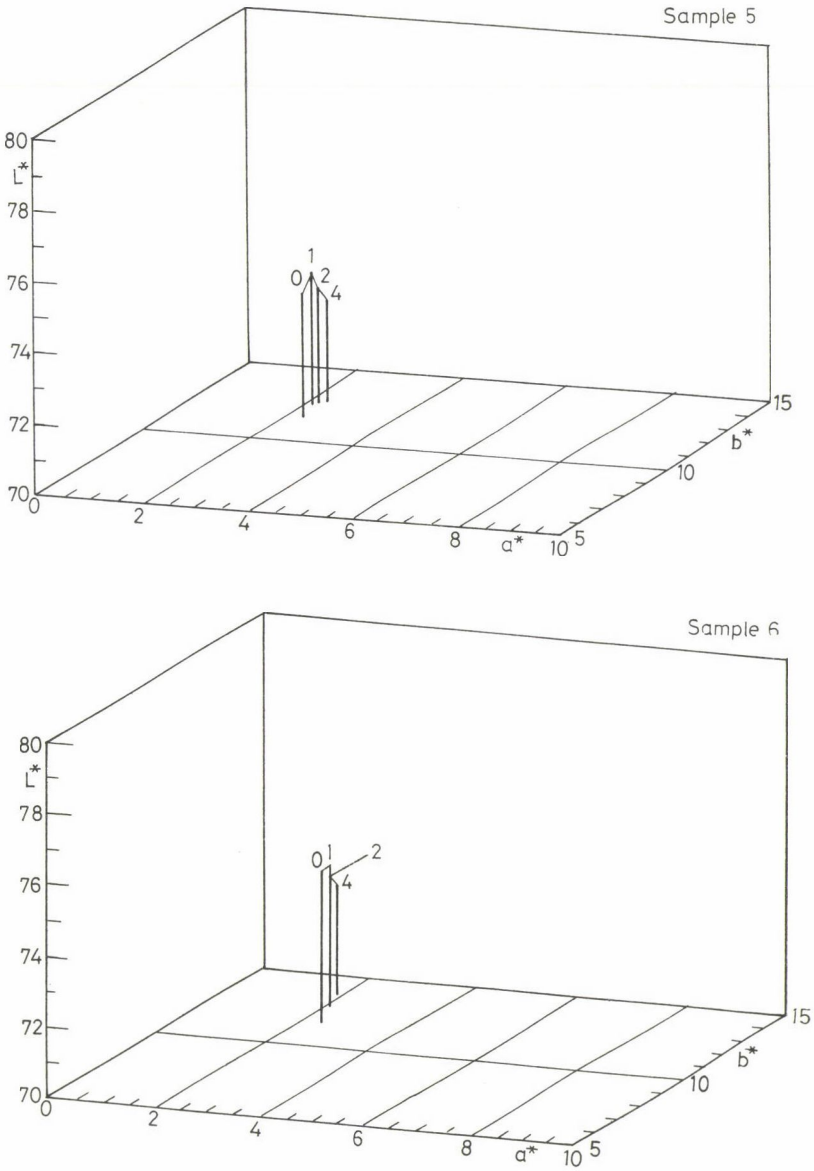


Fig. 2. continued

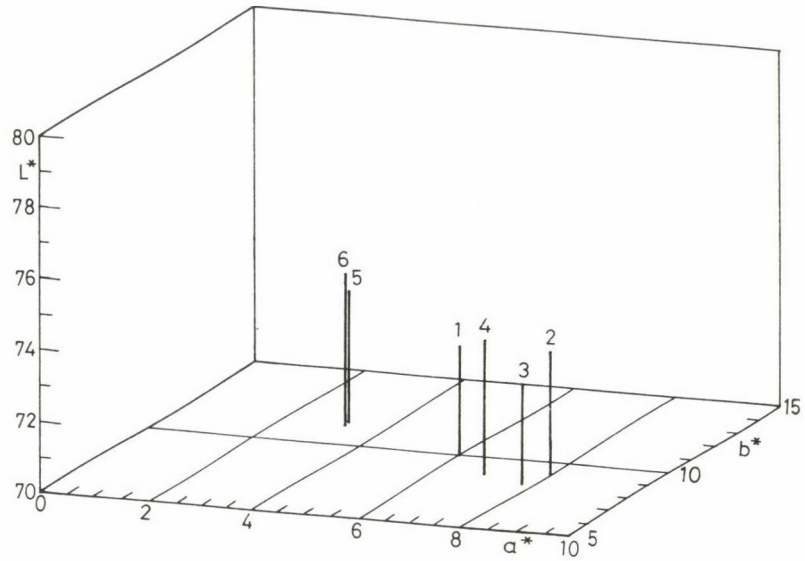


Fig. 3. Colour points of samples stored for 1 week and freshly sliced. For signs of samples see legend to Fig. 1

Here, too, the samples made with glucose and glutamate had the poorest colour. These two samples differed significantly from all other samples. But between the two samples the difference was not significant.

The trend of further changes was similar to that of the samples tested after production.

2.3.2.2. Change of surface colour with time — Change of colour (Tables 2, 3, 4, 5) upon illuminating the slices cut from samples prepared with different additives are analogous to measurements carried out directly after production, therefore these were not illustrated.

2.4. Results of sensory colour evaluation

Results of the sensory ranking of the samples according to colour carried out simultaneously with the instrumental measurements are summarized in Table 6.

The samples prepared with glucose and glutamate were scored significantly lower in the sensory evaluation of slices directly after cutting than the samples containing ascorbic acid or commercial mixture. According to the sensory evaluation the control sample and sample No. 4 did not differ significantly from the samples containing glucose or glutamate. The sample containing commercial mixture additive maintained the colour difference towards the

Table 6

Summary of the results of ranked sensory colour evaluation

Sample	On the day subsequent to preparation				After one week cold storege			
	Time of exposition (h)							
	0	1	2	4	0	1	2	4
1.	abc	ab	ab	ab	abc	abc	ab	b
2.	ab	ab	ab	ab	ab	ab	a	ab
3.	a	a	a	a	a	a	ab	a
4.	abc	ab	ab	ab	abc	abc	ab	ab
5.	c	b	b	b	c	c	b	ab
6.	c	b	b	b	c	c	ab	ab

Legend: Samples in the same column were compared at a time. The sample having the lowest rank sum, in other words the best colour was marked "a", "b" second best and "c" worst. The samples marked with the same letter did not differ significantly from one another.

samples with glucose or glutamate when tested the day after production during 4 h exposition. When, however, scored after one week cold storage the difference was significant only initially and after the first hour exposure to air and light.

The mean values of colour sensation measurements as a function of exposure time are shown in Fig. 4.

Thus, of the additives used, commercial mixture and ascorbic acid resulted in a colour the red character of which as measured instrumentally, was much higher and the yellow character substantionally lower in comparison to that of the control samples. The effect of the mixture of additives was similar to that of commercial mixture and ascorbic acid, only substantially weaker.

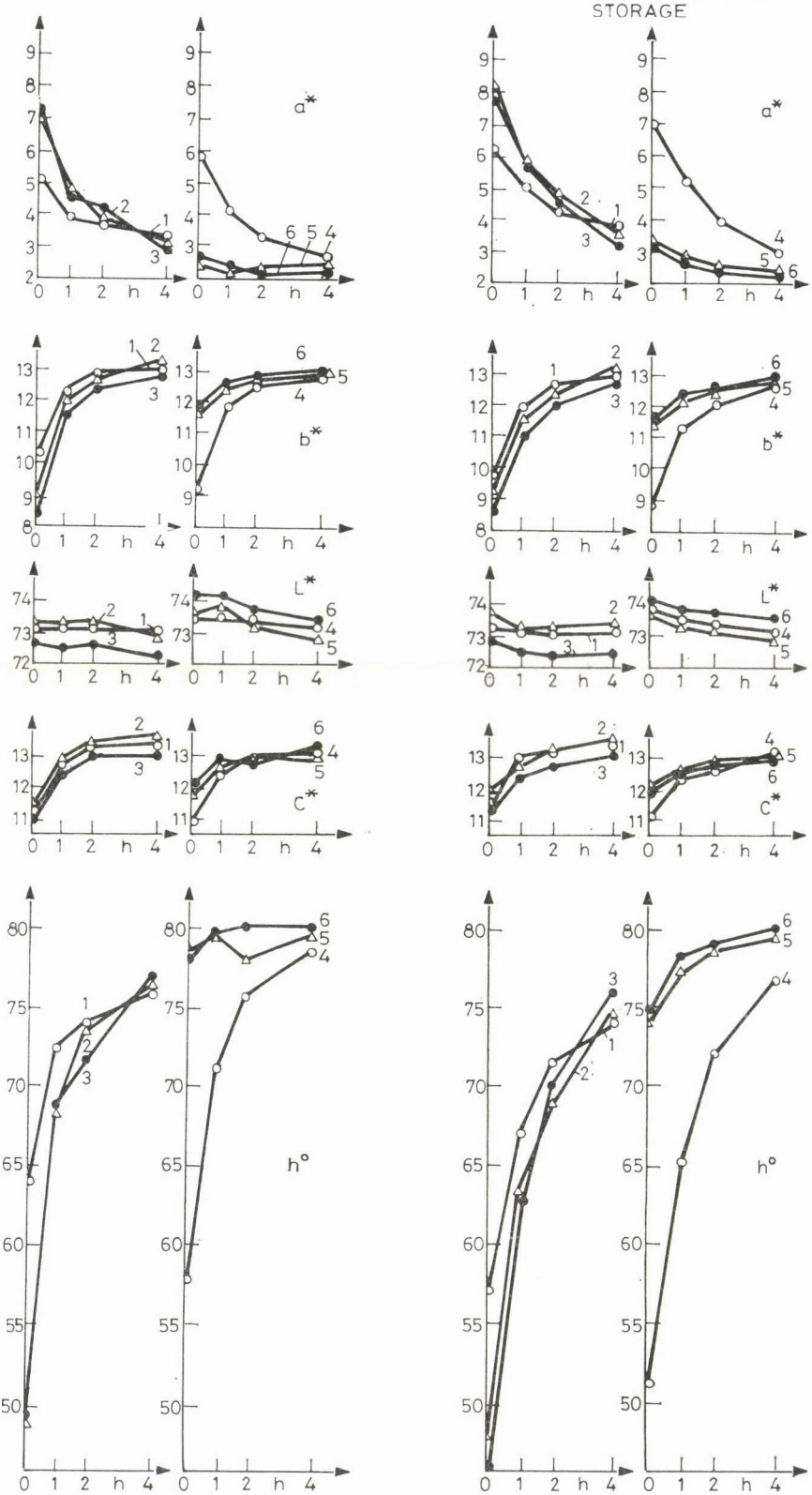
The addition of glucose and glutamate decreased the red character while increasing the yellow character in comparison to the control samples. The negative effect of the glutamate additive is in contradiction with the findings of ANDO (1974) who mentions glutamate among the endogen factors of low molecular weight which improve the colour formation in cured cooked meat products.

The additives promoting colour formation did not exert a colour stabilizing effect in the meat products inasmuch the reduction of the red colour component during exposure to light and oxygen was not slowed down, on the contrary it was accelerated in comparison to that of the control sample.

Due to the higher initial red colour content of the samples with commercial mixture and of ascorbic acid, they had a substantially better colour than the control sample even 1 h after being sliced. The sample containing the mixture of additives was very similar to the control as regards red and yellow

FRESHLY PREPARED SAMPLES

SAMPLES AFTER 1 WEEK COLD STORAGE



colour characters after 1–2 h. After an exposure period of 2 to 4 h none of the samples prepared with additive had a higher red colour character content than the control.

The lightness factor was almost always the lowest in the sample containing commercial mixture. The samples containing ascorbic acid did not differ in this respect from the control while the lightness factor was consistenly the highest in the sample containing glutamate. In comparing data in Tables 2–5 to those in Table 6 (sensory evaluations), it appears that the differences instrumentally established were not visually perceptible by the sensory panel.

2.5. Measurement of the pigment content

The solvent extracts of the meat pigments were measured by spectrophotometry. The results of measurements of the absorption maxima at 540 nm are summed up in Table 7.

Since the nitroso pigment extracted in aqueous acetone was converted to HCl-acidified hematin by adding hydrochloric acid to the extract prior to

Table 7

The total and nitroso pigment content

Sample (additive)	$A_{540nm} \times 10^3$ Nitroso pigment 0	2 h	$A_{540nm} \times 10^3$ Total pigment 0	2 h	Nitroso/total pigment 0	2 h
The day after processing						
1	52	20	142	145	0.37	0.14
2	52	44	129	132	0.40	0.33
3	70	35	140	134	0.50	0.26
4	50	29	130	134	0.38	0.22
5	39	23	130	138	0.30	0.17
6	27	19	131	133	0.21	0.14
After 1 week cold storage at 5 °C						
1	40	27	122	115	0.33	0.23
2	79	38	123	123	0.64	0.31
3	72	43	109	115	0.66	0.37
4	61	31	119	116	0.51	0.26
5	42	11	123	120	0.34	0.09
6	30	17	113	137	0.27	0.12

For samples see Table 1

Fig. 4. Colour stimulus characteristics of samples prepared with various additives. For samples see Fig. 1

photometering (GANTNER, 1959/60) the absorption spectrum obtained was of the same character as that of the total pigment extract in acetone acidified with hydrochloric acid. Thus, the relative concentration of pigments can be directly characterized by the absorption. (The quotient of absorptions as measured at 512 nm and 640 nm was not higher than 2.0, showing that the oxidation of nitroso heme to hematin was complete (HORNSEY, 1956).

Since the pigment extraction tests were not repeated they were only of exploratory character. In spite of this, the results are in good agreement with those of instrumental colour measurement and sensory colour tests.

It is noteworthy, however, that the nitroso pigment proportion is rather low. This is probably due to the fact that the pH of the product, in order to improve its water binding capacity, is set relatively high (Table 1) and this is unfavourable for the conversion of myoglobin into nitroso compound (WIRTH, 1977, 1989). The nitroso proportion was highest in the samples containing commercial mixture additive or ascorbic acid, while the samples containing glucose or glutamate had the lowest nitroso pigment/total pigment proportion. The red colour improving and metmyoglobin formation inhibiting effect of ascorbate and erythorbate is well-known (WIRTH, 1977; 1989; KÖRMENDY & VIRÁGH, 1972; VADA-KOVÁCS et al., 1988; FOX et al., 1967; LIN et al., 1980). The results of our investigations are in harmony with the above findings.

After 2 h exposition of the slices to light and oxygen the total pigment content was not reduced but the nitroso pigment was substantially reduced. These changes are in close correlation with the results of the colour measurements.

In the case of continuous reduction of the nitroso pigment concentration the disturbing affect of metmyoglobin in the spectra may get steadily stronger (GANTNER, 1959/60). Therefore, it cannot be excluded that with advancing exposure to light and oxygen the actual nitroso pigment concentration is lower than expected on the basis of spectrophotometric data. The picture of the conversion process would be, therefore, more explicit if it were followed up by reflection spectrophotometry.

During the one week cold storage the nitroso pigment proportion of the total pigment content increased to a greater or lesser extent in all the samples except the control. This seems to confirm that the characteristic changes in the red colour sensation continue during storage.

The comparison of samples immediately upon slicing and after 2 h standing shows the same as the data of colour measurement, namely that additives of positive effect promote only nitroso pigment formation and does not slow down the deterioration of the nitroso pigments as caused by exposure to light and oxygen.

The factors promoting nitroso pigment formation are, however, still of decisive importance as regards visual stability of colour, because as shown by

HORNSEY (1957), the highly pigmented surface appears still as being red even after the loss of 50% of its pigment content, while initially poorly pigmented, pale surfaces at identical loss of colour appear much more faded.

*

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ESTIMATION OF LIPOIC ACID IN BREWERS' YEAST BY THIN-LAYER AND CAPILLARY GAS CHROMATOGRAPHIC METHODS

E. KOZMA-KOVÁCS, A. HALÁSZ, GY. HAJÓS, A. SASS and F. BOROSS

Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary

(Received: 6 March 1990; revision received: 16 January 1991; accepted: 17 January 1991)

For qualitative determination of lipoic acid (6,8-thio-octanoic acid) a multi-stepwise, two-dimensional thin-layer chromatographic method has been elaborated. Because the large quantity of similar type compounds — mainly fatty acids — interfered, we have developed capillary gas chromatographic methods too.

In both processes lipoic acid — mainly bound to proteins — was liberated by acidic hydrolysis, followed by extraction. Thin-layer chromatography was carried out with samples taken directly from the lipoic acid containing extract.

For gas chromatography the solvent was removed from the extract and its methyl ester derivative was used for analysis. A Supelcowax 10 (of 10 μm thickness) coated glass capillary column of 30 m length, 0,75 mm inner diameter, was used at 240 °C (isotherm conditions), applying H_2 as carrier gas (t_{CH_4} = = 1.25 min). The apparatus used was Hewlett–Packard type 5720 GLC provided with flame ionization detector. Quantitative evaluation was carried out by a HP 3392 Reporting Integrator, calibrated with a known quantity of lipoic acid. The gas chromatographic method was suitable for the estimation of lipoic acid quantities in ppm amounts present in brewers' yeast. Of the known amount of added lipoic acid the total recovery was found to be 30%. The lipoic acid content as determined from different brewers' yeasts (fresh, spray-dried, autolysate) amounted to 1–2.5 μg per g; taking into account the mere 30% recovery, the values come up to 3.3–8.3 μg per g.

Keywords: brewers' yeast, bakery yeast, 6,8-dithio-octanoic acid

Lipoic acid was first isolated by REED and co-workers (1951) from yeast and it was established that it played an important role — as coenzyme — in the oxidation of alfa-keto-acids.

Since then it has been described by many authors (SHIH, 1979; SHIH & STAINSBERGER, 1981) that lipoic acid was present in most cells and it functioned in the alfa-keto dehydrogenase complex.

WADA and co-workers (1961) fed test animals with lipoic acid and lipoic acid amide. Both compounds were easily discharged by the digestive tract and left the body in an oxidised form by the urine. It was also found that lipoic acid and lipoic acid amide secretion increased in rats administered carbon tetrachloride (a potent liver toxin) or suffering from diabetes, as compared to healthy rats.

The lipoic acid is an important compound of pharmaceutical effect due to its free radical binding capacity. That is why we have dealt with the possibilities to determine it in brewers' and bakers' yeast. The lipoic acid is mainly bound to proteins therefore it is absorbed very well giving a better pharmaceutical effect.

HERBERT and GUEST (1975) estimated the lipoic acid content of various microorganisms.

It was found that *Saccharomyces cerevisiae* contained very little lipoic acid if it was cultivated on a medium of 2% glucose content. But the lipoic acid content was substantially higher if the glucose concentration in the medium was only 0.1%. Lipoic acid concentration increased (18%) if pyruvate, cysteine or methionine was added during incubation.

WHITE (1980; 1981) carried out experiments mainly with *E. coli* but he also examined the lipoic acid content in *Saccharomyces cerevisiae*. He carried out hydrolysis according to HERBERT and GUEST (1975) and then applied

Fig. 1. Chromatogram showing the calibration of lipoic acid standard. Start point 1: Standard lipoic acid 10 μg. Start point 2: Standard lipoic acid 20 μg. Start point 3: Standard lipoic acid 30 μg. Start point 4: Standard lipoic acid 40 μg. Thin-layer: Kieselgel 60 F_{254} (Merck). Developing solvent: butanol–ammonia solution (25%)–ethanol (80 : 10 : 10). Detecting reagent: 96% acetic acid containing 1% chromiumotrioxide (VÁGUJFALVY & SZENTE, 1982)

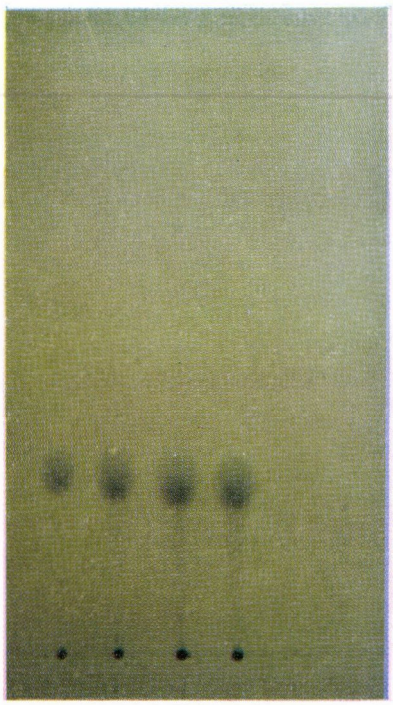

Fig. 2. Chromatogram showing the calibration of the lipoic acid standard. Conditions of thin-layer chromatography correspond to those in Fig. 1 only the detecting is different. Detecting reagent: 96% ethanol containing 5% molybdophosphoric acid (10 min at 110 °C) (STAHL, 1967)

methylenechloride extraction. Thereby the lipoic acid was converted into methyl ester then the disulfide bridge was reduced and it was reacted with benzyl chloride. Thus, gas chromatography (connected to mass spectrometer) was carried out with methyl-6,8-bis-(benzylthio-)-octanate.

In agreement with the findings of HERBERT and GUEST (1975) it was found that the lipoic acid content of *E. coli* depended on the carbon source in the culture medium. Lipoic acid content increased if amino acids were added to the medium and decreased in the presence of glucose. A close positive correlation was observed between lipoic acid and the sum of the specific activity of pyruvate and the α-keto glutarate complex. Both of these enzymes are known to contain lipoic acid.

MUAYAD (1983) used a microbiological method to determine the lipoic acid content in *Saccharomyces carlsbergensis* and *Candida guilliermondii*.

1. Materials and methods

1.1. Materials

Spray-dried brewers' yeast *(Saccharomyces carlsbergensis)*, purified, sifted and washed with water:

Protein content 52%, nucleic acid content 8%

(air-dried sample from the Borsod Brewery).

Brewers' yeast *(Saccharomyces carlsbergensis)* autolysate, salt-free, lyophilized: A suspension of brewers' yeast made in water (10–15%), pH set at 5, was shaken for 16 h at 50 °C, without additive. The enzymes were inactivated at 85 °C for 10 min. The supernatant was separated from the cell mass by centrifuging. It was dried by lyophilization.

Protein content: 65%.

Nucleic acid: 10%.

Fresh brewers' yeast after the fourth plant fermentation *(Saccharomyces carlsbergensis)*: Centrifuged on Beckman model J2–21 for 20 min (10 000 r.p.m.).

Protein content: 16%.

Nucleic acid: 0.3.

Dry matter: 30%.

Bakers' yeast *(Saccharomyces cerevisiae)*:

Protein content: 16.5%.

Nucleic acid: 0.4%.

Dry matter: 30%. From the grocery.

1.2. Methods

1.2.1. Preparation of the lipoic acid containing extract. The first step is the acidic hydrolysis in order to liberate lipoic acid bound to protein according to HERBERT and GUEST (1975).

The acidic solution was filtered and extracted three times with 100 cm^3 dichloromethane. The dichloromethane extracts were dried over anhydrous Na_2SO_4 then under reduced pressure it was concentrated. The concentrates were used for thin-layer chromatography.

1.2.2. Thin-layer chromatography. Since lipoic acid is related to fatty acids their chromatographic properties are akin, too. In the analysis of lipoic acid the same adsorbants, developing agents and detection reagents can be used as for fatty acids.

1.2.2.1. The calibration of the thin-layer chromatographic system by lipoic acid standard. The calibration by lipoic acid standard is shown in Figs. 1 and 2.

The two chromatographic methods differ only in the detection reagents used. In Fig. 1 the chromatogram was detected with 96% acetic acid containing 1% chromium trioxid. This reagent was used by VÁGUJFALVI and SZENTE

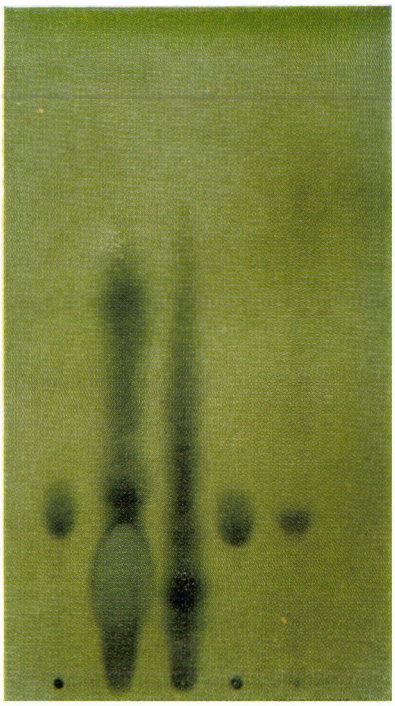

Fig. 3. Chromatography of isolated brewers' yeast fractions. Start points 1 and 4: Lipoic acid standard (5 μg). Start point 2: Extract of spray-dried brewer's yeast, 2nd fraction (20 μl). Start point 5: Extract of spray-dried brewers' yeast, fraction 3 (20 μl). Start point 3: Autolysate of brewers' yeast, fraction 2 (20 μl). For conditions of chromatography see in Fig. 2

(1982) according to LANGLER (1975) to detect thiols, sulfides and sulfinates. On the chromatogram layer the blue lipoic acid spots appear on a yellow background in about 15 to 30 min after application. After a longer period the background becomes darker. The chromatogram in Fig. 2 was detected with 96% ethanol containing 5% molybdophosphoric acid. This reagent was recommended by STAHL (1967) in the thin-layer chromatography of fatty acids.

The adsorbent was Kieselgel 60 F_{254} (Merck). The mixture of butanol – ammonia (25% in water) – ethanol (96%) (80:10:10) was used as developing solvent (SHIH & STEINSBERGER, 1981).

Of the standard lipoic acid 5 mg cm^{-3} dichloromethane solution 2, 4, 6 and 8 μl doses, (10, 20, 30, 40 μg respectively) were applied to the thin-layer.

With the chromium trioxide detection quantities below 10 μg could hardly be detected.

With the molybdophosphoric acid detection 5 μg lipoic acid could be evaluated.

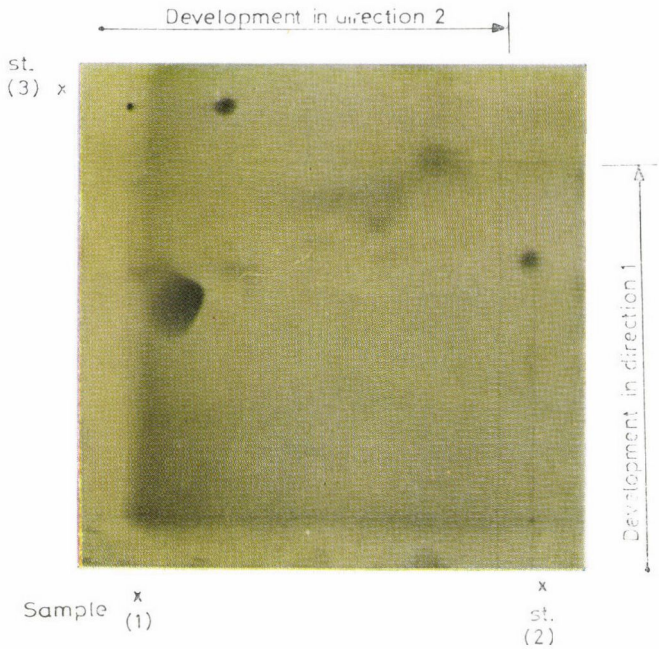

Fig. 4. Two-dimensional thin-layer chromatography of the extract obtained from spray-dried brewers' yeast. Start point 1: Extract of spray-dried brewer's yeast (30 μl). Start point 2: Lipoic acid standard (5.0 μg). Start point 3: Lipoic acid standard (5.0 μg). Developing solvent, used for first direction: chloroform–methanol–formic acid (80 : 10 : 10). Developing solvent used for second direction: butanol–ammonia solution (25 %)–ethanol (80 : 10 : 10). Detecting solvent: 96 % ethanol containing 5 % molybdophosphoric acid (10 min at 110 °C)

1.2.2.2. Chromatographic step to clean up lipoic acid — The extracts, made of the yeast samples, could not be satisfactorily separated by the mentioned methods.

Therefore, the fraction of the extract containing the lipoic acid was enriched by thin-layer chromatography.

The adsorbent and the detecting reagent are the same as for the chromatogram in Fig. 2. The solvent applied was benzene — petroleum ether (40–70 °C) — acetic acid mixture (80:12.5:7.5) as used for the separation of fatty acids (STAHL, 1967).

On the basis of the R_f value of the standard lipoic acid three fractions were isolated: spots below the R_f value of lipoic acid (fraction 1); spots eluted in the same location as lipoic acid (fraction 2); spots adsorbed above the R_f value of lipoic acid (fraction 3). The fractions were eluted with dichloromethane and concentrated in N_2 gas stream.

1.2.2.3. Rechromatography of the isolated fractions — Chromatography was applied again to the three fractions. Thin-layer chromatography conditions were the same as for the lipoic acid standard, shown in Fig. 2.

The lipoic acid standard was applied to the thin-layer (start point 1 and 4); extract of spray-dried brewers' yeast, fractions 2 and 3 (start point 2 and 5); extract of brewers' yeast autolysate, fraction 2. Separated fractions are shown in Fig. 3.

It can be seen that all the samples applied appear at the appropriate R_f value of lipoic acid. Because of the difficulty in separating the fractions two-dimensional thin-layer chromatography was applied.

1.2.2.4. Two-dimensional thin-layer chromatography — For the two-dimensional chromatography Kieselgel 60 F_{254} (Merck) thin-layer was used.

Development in the first direction was carried out with the solvent chloroform – methanol – formic acid (80:10:10) by which a higher R_f value could be achieved.

Development in the second direction was carried out with butanol – ammonia solution (25% in water) — ethanol (96%) (80:10:10) thereby obtaining a lower R_f value. The two developing solvents were used by SHIH and STEINSBERGER (1981) to develop chromatograms in succession from lipoic acid extracts. The separation is shown in Fig. 4.

The thin-layer with the sample and standard applied was developed in the first direction to the marked height. After drying the thin layer was turned away by 90 degree using the developed spots as starting points and in a 3rd point was again standard applied and then the second developing solvent was used in the second direction.

On the basis of the R_f values of the two lipoic acid standards three spots appeared representing three substances adsorbed very near to one another (two stronger and one very pale spot). Compared to the standard R_f value the pale spot corresponds to lipoic acid.

1.2.3. Capillary gas chromatography. Since the multi-step thin-layer chromatography is not suitable for the convincing evaluation of lipoic acid, a capillary gas chromatographic method was developed.

To be able to carry out the analyses the methyl ester of the standard lipoic acid and extracts of the samples, were prepared. From the sample free of dichloromethane 30–60 mg was weighed and this was used to prepare the methyl ester according to KINSELLA and co-workers (1977) and to the micro-method of LÁSZTITY (1978).

The analyses were carried out on the Hewlett Packard 5720 gas chromatograph fitted with a flame ionization detector. The quantitative evaluation was carried out on the HP 3392 Reporting Integrator connected to the gas chromatograph, based on calibration with standard lipoic acid of a known quantity.

Conditions of chromatography were as follows:

A glass capillary column of 30 m length, 0.75 mm inner diameter, coated with 10 μm thickness Supelcowax 10.

The carrier gas: H_2 at linear rate $t_{CH_4} = 1.25$ min.
Temperature program: Isothermal at 240 °C.
Temperature of injector port: 240 °C.
Temperature of detector: 260 °C.

2. Results

A multi-step, two-dimensional thin-layer chromatographic method was developed for the qualitative determination of lipoic acid.

The aim of carrying out thin-layer chromatography in succession was the purification of lipoic acid containing extract. Fatty acids and other lipid

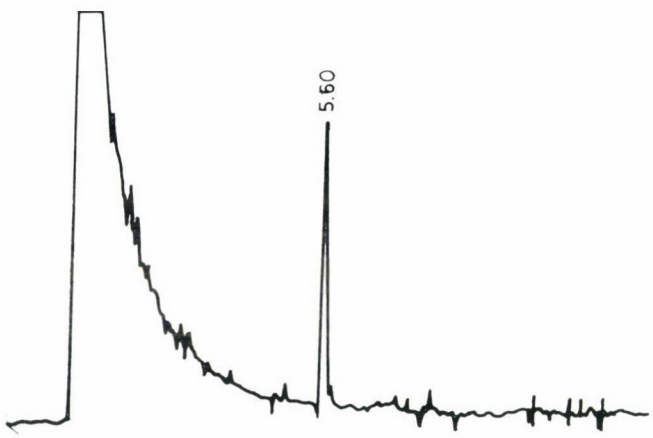

Fig. 5. Gas chromatogram of 0.2 μg standard lipoic acid methylester. A glass capillary column of 30 m length, 0.75 mm diameter with 10 μm thick Supelco-wax 10 coating. Temperature program: 240 °C isotherm

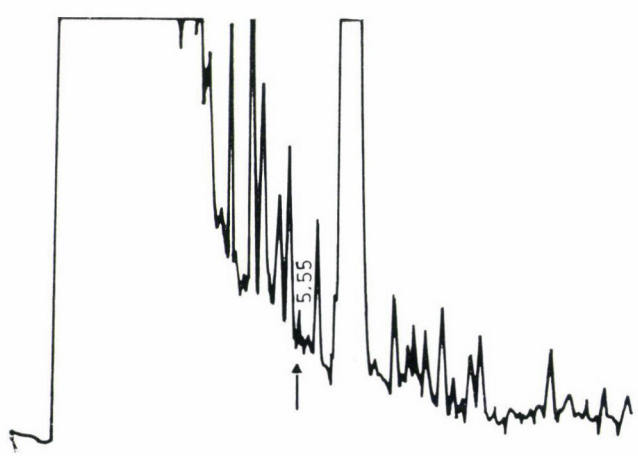

Fig. 6. Gas chromatogram made of the extract of spray-dried brewers' yeast. For details see legend of Fig. 5

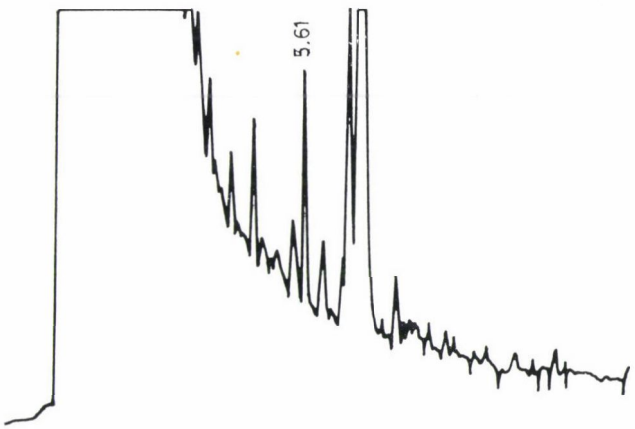

Fig. 7. Chromatogram of spray-dried brevers' yeast with added lipoic acid standard. For details see legend of Fig. 5

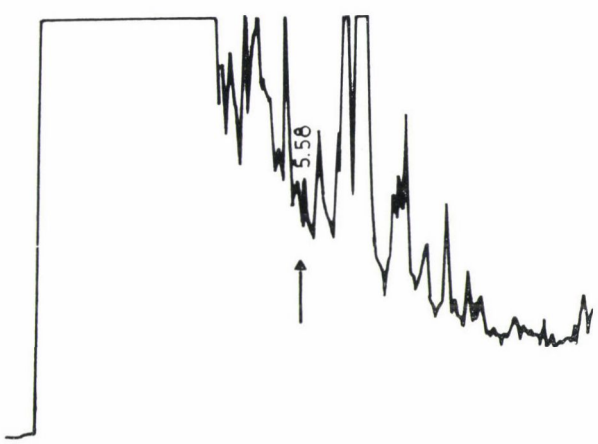

Fig. 8. Gas chromatogram of brewers' yeast autolysate. For conditions of gas chromatography see legend to Fig. 5

components could be removed only by two dimensional thin-layer chromatography.

The capillary gas chromatographic method developed is suitable for the determination of ppm quantities of lipoic acid.

Results obtained by capillary gas chromatography are shown in the following figures.

Gas chromatogram of 0.2 μg standard lipoic acid methyl ester is shown in Fig. 5. The retention time is 5.60 min.

Figure 6 illustrates the gas chromatogram of the extract obtained from the spray-dried brewers' yeast. Lipoic acid is marked on the chromatogram (the retention time is 5.55 min).

Table 1

Lipoic acid content of the samples determined by capillary gas chromatography

Microorganisms	μg per g spray-dried sample 30% recovery taken into account
Saccharomyces carlsbergensis:	
spray-dried	2.6 ± 0.21
autolysate	8.3 ± 0.15
after 4th fermentation	4.0 ± 0.39
Saccharomyces cerevisiae:	
fresh sample	3.3 ± 0.46

(Each result is the average of three parallel determinations)
The *Saccharomyces carlsbergensis* samples originated from different fermentations.

Figure 7 presents the chromatogram of standard lipoic acid added to the sample (spray-dried brewers' yeast). The peak of 5.61 min became higher proving that the presumed peak is really that of lipoic acid.

The gas chromatogram in Fig. 8 is obtained from the extract of brewers' yeast autolysate (the retention time is 5.58). The previous defatting of sample extract has not been needed because of the great resolution of the capillary column.

Results are summarized in Table 1. They are the averages of three parallel determinations.

The lipoic acid content of the samples varied between 0.8 and 2.5 μg per g. The highest quantity was obtained from the autolysate of brewers' yeast *(Saccharomyces carlsbergensis)*. It should be noted that the samples were independent from one another and were produced at different times.

Table 2

Lipoic acid content of different microorganisms
(data from the literature)

Authors	Microorganisms	Methods	Lipoic acid content (μg per g dry matter)
HERBERT & GUEST (1975)	*S. cerevisiae*	Turbidimetric, Polarographic	1.0 8.5
WHITE (1981)	*S. cerevisiae*	GLC	0.16[a]
MUAYAD ALI KOJI (1983)	*S. carlsbergensis*	Microbiological	9.1
	Candida guilliermondii	Microbiological	3.5

[a] Related to wet sample.

If it is taken into consideration that 70% loss is caused by sulphuric acid hydrolysis in the lipoic acid added to the samples, the results obtained correspond to 2.6–8.3 μg per g. Related data from the literature are presented in Table 2. This table contains data for bakers' yeast (*Saccharomyces cerevisiae*) too, because only few data were available for brewers' yeast (*Saccharomyces carlsbergensis*) and *S. guilliermondii*.

There is a close agreement between the literature data and our results (1.0–9.1 μg lipoic acid per g spray-dried yeast and 2.6–8.3 μg lipoic acid per g spray-dried yeast, respectively) although there may have been a difference in the samples.

3. Conclusions

The multi-step thin-layer chromatographic method is not recommended for the practical examination of lipoic acid containing a big quantity of disturbing fatty compounds.

The gas chromatograph fitted with high resolving power capillary column enabled us to investigate the samples without defatting. Lipoic acid was studied as its methylester derivative. Because the lack of S-selective detector neither the reduced form of lipoic acid (dehydrolipoic acid) nor the shorter chain analogues could be determined.

The samples prepared by different techniques from brewers' yeast and the fresh bakers' yeast had relatively low lipoic acid content. To increase the lipoic acid formation the composition of the nutrient medium has to be changed taking into account the recommendations of HERBERT and GUEST (1975) and WHITE (1980; 1981), according to which the presence of amino acids increases, while that of glucose reduces the lipoic acid content. Presumably the addition of octanoates, according to PARRY (1977) and essential fatty acids given by CARREAU and co-workers (1977) also leads to increased lipoic acid formation.

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VOLATILE FLAVOUR COMPONENTS OF GARLIC ESSENTIAL OIL

J. PINO[a], A. ROSADO[a] and A. GONZALEZ[a]

[a]Research Institute for Food Industry (IIIA) Rancho Boyeres Km 3 1/2, P. O. Box 13400, Havana. Cuba
[b]National Center for Scientific Researches, Havana. Cuba

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The essential oil of garlic was investigated by means of HRGC, GC-MS and by eluate sniffing techniques. Fifteen compounds were identified including the following new ones: (Z)- and (E)-2-propenyl 1-propenyl disulfide, 1,2,3-trithi-4-ene and 2-propenyl tetrasulfide. By determination of the flavour dilution (FD) factor, which is proportional to the aroma value, it was found that di(2-propenyl) trisulfide, di(2-propenyl) disulfide and methyl 2-propenyl trisulfide are the most potent flavour compounds in the essential oil. The FD factor was also used for the approximation of odour values of the new compounds.

Keywords: volatile components, flavour, garlic

Garlic (*Allium sativum* L.) is greatly appreciated throughout the world because of its many uses in medicine and as flavouring of food products. Originating from the Eastern mediterranean countries, this liliacea spread rapidly throughout Europe, Asia and America.

Many works have been devoted to its essential oil and these were recently reviewed by several authors (VAN STRATEN & MAARSE, 1983; SHANKARAMARAYANA et al., 1982; FENWICK & HANLEY, 1985; CARSON, 1987).

The flavour of garlic results when the vegetable is crushed or bruised, and sulfur-containing amino acids are enzymatically decomposed to yield volatile sulfur-containing compounds. Many unexpected reactions occur, and many new and unusual compounds have been isolated. Nevertheless, the volatiles which have been identified in garlic oil are surprisingly few, especially when compared with other species of genus Allium — onion, leek and shallot. This is mainly due to the fact that secondary reactions, yielding a complex mixture of volatiles which result from cooking or processing of the other Allium species, are generally absent in the garlic samples which have been examined (BOELENS et al., 1971). Furthermore, garlic contains little (E)-S-(1-propenyl)-L-cysteine sulfoxide which in the case of other Allium species, produces many oxygen-containing volatiles (FENWICK & HANLEY, 1985).

The following investigation was undertaken to identify the major volatile compounds and their flavour contribution in garlic essential oil. For this purpose, the volatile components separated from the essential oil were analysed by high resolution gas chromatography (HRGC), combined gas chromatoggraphy – mass spectrometry (GC-MS) and by eluate sniffing.

1. Materials and methods

1.1. Materials

Garlic essential oil distilled at a pilot plant during the summer of 1988 was used in this study. The essential oil was drawn from the bulked product obtained by the steam distillation of 500 kg of field-run garlics of mixed varieties, maturities and sizes.

1.2. Instrumental analysis

HRGC was performed with a Pye Unicam 204 gas chromatograph. The thin-film capillary columns used were OV-101 (30 m × 0.25 mm, fused silica) and PEG 20M (25 m × 0.25 mm, fused silica). The temperature was programmed: 60 to 200 °C at 4 °C min^{-1}, then 10 min isothermal. The flow rate of the carrier gas hydrogen was 0.6 cm^3 min^{-1} and of the auxiliary make-up gas was 20 cm^3 min^{-1}. At the end of the capillary column, the effluent was split 1 : 1 to a flame ionization detector and a sniffing port (the end of a stainless steel tubing 20 × 0.8 cm), both held throughout at 200 °C. Air saturated with water was passed through the stainless steel tubing at 300 cm^3 min^{-1}.

Retention data of compounds are presented as Kovats' indices (KI) according to Van den Dool and Kratz (1963).

GC-MS analyses were performed using a Jeol DK 300 with an OV-101 capillary column. The conditions of the GC were the same as reported above, excepting the flow rate of the carrier gas helium which was 0.8 cm^3 min^{-1}. The ion source temperature was 200 °C and electron impact mass spectra were generated at 70 eV.

Quantification of volatile compounds was made by relating individual peak area to total area of the reconstructed ion chromatogram with the aid of a JMA 3100 computer.

1.3. HRGC eluate sniffing and odour intensity evaluation

During the HRGC of the essential oil, the odour active regions of the eluate were evaluated and the aroma notes of these regions were assigned. After stepwise dilution of the oil by the addition of diethyl ether, the HRGC and the eluate sniffing were performed with each diluted sample. The dilution procedure was continued until no odour active region was detected. This procedure was carried out in duplicate.

The odour intensity was evaluated by the flavour dilution (FD) factor. This is the highest dilution at which a compound is still smelled. The undiluted sample has then, by definition, an FD factor of 1. An aromagram is the plot

of FD factors for all odour active compounds in the essential oil against their retention index (KI). The aromagram was evaluated by two judges. This procedure has been previously used for evaluating the odour intensity of flavour compounds (SCHIEBERLE & GROSCH, 1987).

The approximate odour threshold O_x of a compound X was calculated using the odour threshold of a known substance (O_k) and their FD factors and concentrations (C) by the following expression:

$$O_x = \frac{O_k C_x \mathrm{FD}_k}{C_k \mathrm{FD}_x}$$

2. Results

A typical reconstructed chromatogram of the garlic essential oil obtained on an OV-101 capillary column by measuring the total ion current is shown in Fig. 1. The order of elution on the PEG 20M capillary column was different, but no additional components of the essential oil were separated on it. Identifications of the main components by chromatographic and mass spectra data are given in Table 1. Measured mass spectra were compared with those reported in the literature (BRODNITZ et al., 1971; STENHAGEN et al., 1974; TRESSL et al., 1977; VERNIN et al., 1986).

In total, 15 volatile compounds were identified, four of them had not previously been reported in garlic.

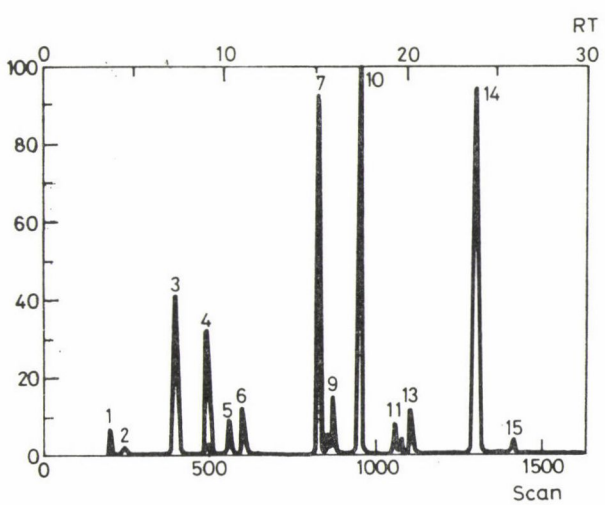

Fig. 1. Reconstructed chromatogram obtained by GC-MS from garlic essential oil on OV-101 capillary column

Table 1

Volatile compounds identified in garlic essential oil

Peak No.[a]	Compound	KI[b]	M+	m/z[c]
1	Methyl 2-propenyl sulfide	797	88	88, 73, 41, 45, 39
2	Dimethyl disulfide	809	94	94, 79, 28, 45, 46
3	Di(2-propenyl)sulfide	862	114	45, 41, 73, 39, 72
4	Methyl 2-propenyl disulfide	897	120	41, 120, 39, 45, 79
5	1,2-Dithiocyclopentene	911	104	103, 104, 45, 39, 71
6	Dimethyl trisulfide	942	126	126, 79, 45, 47, 64
7	Di(2-propenyl)disulfide	1056	146	41, 81, 146, 105, 126
8	(Z)-1-Propenyl 2-propenyl disulfide[d]	1069	146	41, 45, 39, 73, 146
9	(E)-1-Propenyl 2-propenyl disulfide[d]	1073	146	41, 45, 39, 73, 146
10	Methyl 2-propenyl trisulfide	1126	152	87, 73, 41, 45, 39
11	3-Vinyl-1,2-dithi-5-ene	1171	144	111, 144, 97, 45, 71
12	1,2,3-Trithi-4-ene[d]	1196	136	71, 136, 72, 45, 64
13	3-Vinyl-1,2-dithi-4-ene	1206	144	72, 71, 45, 144, 111
14	Di(2-propenyl)trisulfide	1329	178	73, 41, 113, 39, 45
15	2-Propenyl tetrasulfide[d]	1611	170	170, 41, 64, 128, 106

[a] Peak number in Fig. 1.
[b] Kovats indices on OV-101 capillary column.
[c] Main fragments are classified by decreasing intensity order.
[d] Reported for the first time as garlic volatile component.

Components 7, 8 and 9 had molecular weights of 146 and according to mass spectra, all contained 2-propenyl group and two sulfur atoms. The mass spectra of components 8 and 9 were essentially identical (Fig. 2). All three spectra contained characteristic ions of m/z 146 and 105 (loss of 2-propenyl group moiety). Mass spectrum and retention indices for component 7 agreed closely with those of di(2-propenyl)disulfide (MIZUTARI & OBATA, 1971; VERNIN et al., 1986).

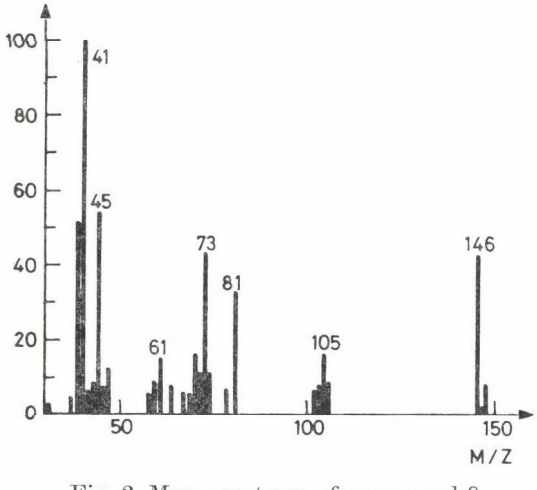

Fig. 2. Mass spectrum of compound 8

The fragmentation of the mass spectra of components 8 and 9 were presumed on the basis of the data of the metastable ion peaks for disulfides and of unsaturated disulfides (BRODNITZ et al., 1969). In Fig. 2 the relative abundance of fragment m/z 73 (loss of 1-propenylthio group moiety) explain this remarkable difference between di(2-propenyl) disulfide and 1-propenyl disulfide. The bond of S-C (2-propenyl group moiety) is more easily cleaved than that of S-C (1-propenyl group moiety), while 1-propenylthio cation (m/z 73) is more stable than 2-propenyl-thio cation. Another difference between the two compounds is the absence of peak 113 (loss of SH radical) in spectra of components 8 and 9, since the release of hydrothio radical (SH) from di(2-propenyl) disulfide is easier than that from 1-propenyl-containing disulfide. From this information the components 8 and 9 are presumed to be (Z)-1-propenyl 2-propenyl disulfide and (E)-1-propenyl 2-propenyl disulfide, respectively.

Component 12 had a molecular weight of 136 according to its mass spectrum (Fig. 3). Also on the basis of the mass spectrum, the compound contained three sulfur atoms and the characteristic ions of m/z 103 (M^+ minus SH) and 71 (M^+ minus S_2H). These evidences suggest that component 12 is presumed to be 1,2,3-trithi-4-ene, a cyclic sulfide not previously reported to occur in Allium species. This sulfide is believed to originate by the elimination of hydrogen sulfide and rearrangement of 2-propenyl tetrasulfide which also had been identified in the essential oil.

The mass spectrum of component 15 is shown in Fig. 4, which had abundant ions of m/z 128 (S_4), 64 (S_2), 41 (2-propenyl group moiety), 138 (M^+ minus S) and 106 (M^+ minus S_2). The molecular weight of 170 suggested that the component is 2-propenyl tetrasulfide. The presence of this compound

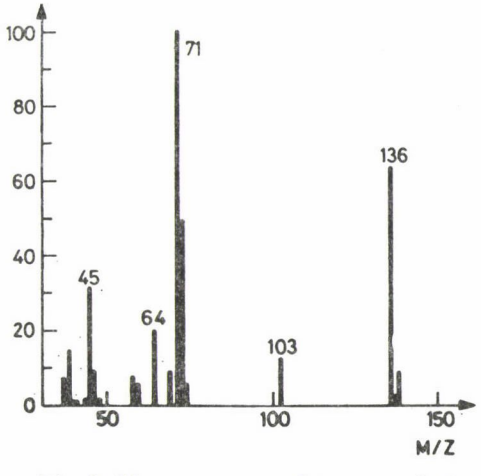

Fig. 3. Mass spectrum of compound 12

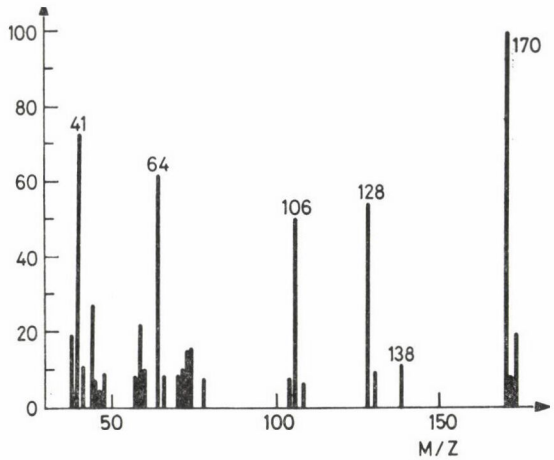

Fig. 4. Mass spectrum of compound 15

is not unexpected since ZOGHBI and co-workers (1984) had isolated methyl 2-propenyl tetrasulfide and di(2-propenyl) tetrasulfide in garlic bush essential oil.

The results of the quantitative analysis and aroma description of individual components are summarized in Table 2. For comparison the percentage composition range of garlic essential oils cultivated in various countries is also presented (VERNIN et al., 1986). Results showed marked differences in

Table 2

Percentage composition and aroma description of volatile compounds identified in garlic essential oil

Peak[a] No.	Compound	Percentage composition		Aroma description
		Calculated	Literature[b]	
1	Methyl 2-propenyl sulfide	4.4	0.4–1.7	Garlic, savory
2	Dimethyl disulfide	0.5	–	Vegetable, cabbage
3	Di(2-propenyl)sulfide	7.9	1.6–10.4	Pungent, garlic
4	Methyl 2-propenyl disulfide	5.6	0.6–7.4	Garlic, savory
5	1,2-Dithiocyclopentens	1.1	–	Fried garlic
6	Dimethyl trisulfide	1.6	0.2–4.4	Onion
7	Di(2-propenyl)disulfide	20.7	29.5–50.5	Pungent, garlic
8	(Z)-1-Propenyl 2-propenyl disulfide	0.7	–	Boiled onion
9	(E)-1-Propenyl 2-propenyl disulfide	1.6	–	Boiled onion
10	Methyl 2-propenyl trisulfide	21.7	4.2–14.4	Garlic, savory
11	3-Vinyl-1,2-dithi-5-ene	0.8	–	Fried garlic
12	1,2,3-Trithi-4-ene	0.2	–	H_2S-like
13	3-Vinyl-1,2-dithi-4-ene	1.4	–	Fried garlic
14	Di(2-propenyl)trisulfide	31.9	30.1–42.0	Pungent, garlic
15	2-Propenyl tetrasulfide	0.3	–	Garlic, savory

[a] Peak number in Fig. 1.
[b] From VERNIN et al. (1986).

composition, particularly in content of methyl 2-propenyl sulfide, di(2-propanyl) disulfide and methyl 2-propenyl trisulfide. The percentages of mixed sulfide and trisulfide (methyl 2-propenyl) were enhanced in the analyzed essential oil, while it contained lower percentage of di(2-propenyl) disulfide.

Changes in the amount of sulfide compounds of garlic essential oil may be attributed either to various precursors or to dismutation reactions of disulfide derivatives during hydrodistillation at atmospheric pressure or GLC analysis. The fact that the di(2-propenyl) disulfide content, a dismutation reaction product, is in agreement with the literature data and that other mixed sulfide and trisulfide compounds are in higher percentages indicates that dismutation reactions take place during essential oil processing or GLC analysis, and not due to lower concentration of the flavour precursor 2-propenyl-thiocysteine S-oxide.

The aroma description of the individual components suggested all of them as imparting characteristic flavour.

The contribution of a flavour compound to the aroma of a food depends on its "aroma value", which is defined as the ratio of the concentration of the compound to its odour threshold (ROTHE, 1976). Since these two factors are

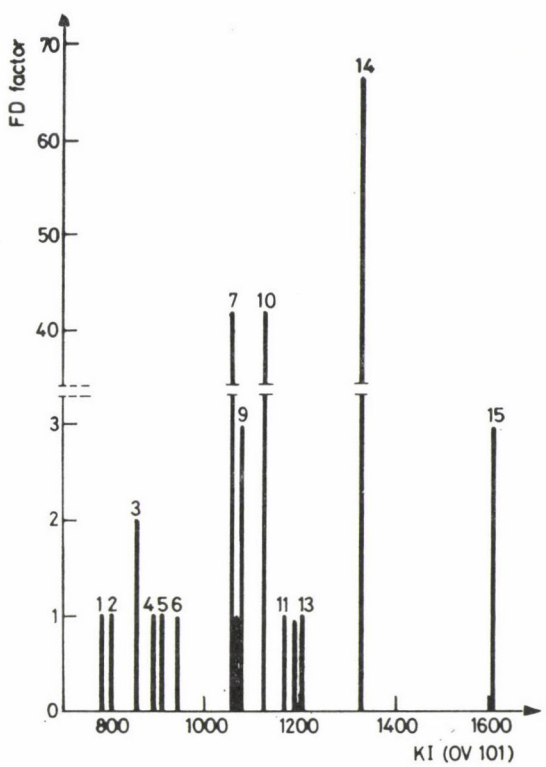

Fig. 5. Aromagram of garlic essential oil. Numbering of components as in Fig. 1

different to determine, the use of FD factors which are proportional to the aroma values may be of great advantage. Figure 5 shows the aromagram of the garlic essential oil. Di (2-propenyl) trisulfide and disulfide, as well as methyl 2-propenyl trisulfide were the most potent flavour compounds, while the remaining components were of lower significance for the flavour of garlic essential oil.

The method for the approximation of the odour threshold value was tested on the new volatile compounds found in garlic (Table 3). Di (2-propenyl) disulfide with an odour threshold in air of 0.0072 ppb (VERNIN et al., 1986) was always used as the reference substances. The lower odour threshold observed for these volatile compounds may prove them of greater significance at higher percentages than actually occurred.

Table 3

Odour threshold values of new volatile compounds found in garlic essential oil

Compound	Approximative odour threshold in air (ppb)
(Z)-1-Propenyl 2-propenyl disulfide	0.003
(E)-1-Propenyl 2-propenyl disulfide	0.008
1,2,3-Trithi-4-ene	0.003
2-Propenyl tetrasulfide	0.001

3. Conclusion

The present investigation illustrates the advantages of applying GC-MS, HRGC and eluate sniffing to research concerning volatile compounds in natural or processed food products. The application of these techniques allowed to identify 15 volatile compounds in garlic essential oil, four of them — (Z)- and (E)-1-propenyl 2-propenyl disulfide, 1,2,3-trithi-4-ene and 2-propenyl tetrasulfide — not previously reported as occurring in garlic. The determination of the FD factor indicate that di(2-propenyl) trisulfide, di(2-propenyl) disulfide and methyl 2-propenyl trisulfide give a fundamental contribution to the garlic essential oil, for intensity and qualitative characteristics.

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THE BIOLOGICAL VALUE OF MAIZE-FENUGREEK FLOUR MIXTURE IN SOME FOOD PRODUCTS

A. EL-KADY[a], R. LÁSZTITY[b], M. HIDVÉGI[b], M. KHALIL OSMAN[c] and L. SIMON-SARKADI[b]

[a]Rice Research and Training Center, Kafr El-Sheikh. Egypt
[b]Technical University, Budapest, H-1111 Budapest, Műegyetem rkp. 3. Hungary
[c]University of Mansoura, Mansoura. Egypt

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The gross chemical and amino acid composition and in vitro nutritional qualities of maize (Balady variety), and fenugreek (*Trigonella foenum* L.; Giza 2 variety) flours, as well as nutritional and organoleptic qualities of some baked products made from mixtures of maize and fenugreek flours vere investigated.

Protein content of baked products made from maize and fenugreek flour mixtures was higher as fenugreek ratio increased. Baking resulted in considerable loss percentages in most essential amino acids especially tryptophan, methionine, cystine, phenylalanine and tyrosine, while glutamic acid, threonine, valine and aspartic acid were the most thermostable amino acids. Baking led to marked losses in chemical scores of all type of baked products. Organoleptic evaluation revealed that the addition of 5% and 8% fenugreek flour in the flour mixtures resulted in acceptable products.

Keywords: fenugreek, maize, biological value, baked goods, enrichment with fenugreek flours

Cereal grains constitute the major energy and protein supply in developing countries (HARPER & HEGESTED, 1974). Wheat is the most widely used cereal in making bread and other baked products. Production of wheat in Egypt, however, is not enough to cover the population's consumption. That is why utilization of maize flour as partial or whole substitute instead of wheat flour in breadmaking has been suggested by some investigators (DENDY et al., 1970; SHEHATA & KHORSHID, 1982; KHORSHID & EL-TALAWI, 1982).

The use of home-made maize bread is well established in Upper Egypt and some localities in the Delta. Addition of protein rich legume such as broad bean or fenugreek to this type of bread has been a common practice since many years. Fenugreek (*Trigonella foenum graecum* L.) is an annual herbaceous plant of the leguminous family, widespread in certain countries of the Mediterranean basin and Asia. The seeds are rich in protein which ranges from 20 to 30% and lysine (JAMALIAN & PELLETT, 1968; EL-MADFA, 1975; EL-MAHDY & EL SEBAIY, 1982; HIDVÉGI et al., 1984). Only little information is available in the literature about the effects of added fenugreek flour on the chemical composition and biological value of maize bread.

In this study, chemical and nutritional evaluations were carried out for maize and fenugreek flours. Mixtures of these two flours were used to make bread, salty wafer and sponge cake, and the nutritional qualities of these products were investigated.

1. Materials and methods

1.1. Materials

Balady maize variety was obtained from Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt. Fenugreek seeds of Giza 2 variety were obtained from the local market at Kafr El-Sheikh, Egypt.

1.2. Methods

Maize grains and fenugreek seeds were cleaned by excluding foreign seeds and materials, then ground to a fine powder by a laboratory mill and sieved through 250 μm sieve.

Moisture, protein lipids and ash contents were determined using the methods described in A.O.A.C. (1975). Protein was calculated as $N \times 6.25$. Digestibility of maize and fenugreek proteins were measured by using pancreatin and trypsin enzymes according to the method described by Hsu and co-workers (1977). The digestibility was expressed as percent of total protein.

Amino acids composition was determined after acid hydrolysis of ground samples using automatic amino acid analyser (type AAA 881 Mikrotechna Praha) as described by Spackman and co-workers (1958). Tryptophan was photometrically determined in the alkaline hydrolysates of ground samples using the method reported by Örsi (1985).

In vitro biological values were calculated according to the methods reported by Mitchell and Block (1946); FAO/WHO (1973); Mørup and Olesen (1976).

The utilizability of maize and fenugreek flours in making three different types of baked products were tested as follows:

— Breadmaking: Fenugreek flour was introduced at three different levels as illustrated in Table 1.

The technological process and baking tests were carried out according to the Egyptian baking technology reported by Refai (1966).

— Salty wafer: Two formulae were used. Ratios of maize flour to fenugreek flour were 95 : 5 and 90 : 10 g. The other components were 3 g salt and 100 cm^3 wafer. Twenty five to thirty grams of batter were baked in a baking pan using thin layer of oil.

Table 1

Formulae used in making maize–fenugreek bread

Components	Quantity (g)		
Maize flour	95	90	80
Fenugreek flour	5	10	20
Salt	3	3	3
Yeast	5	5	5
Water	100	100	100

— Sponge cake: The components used in making the sponge cake were 150 g eggs, 67 g maize flour, 8 g fenugreek flour, 60 g sugar, and 3 g baking powder. Baking was carried out at 185 °C for 24 minutes as recommended by CHARLEY (1956).

Organoleptic qualities of the baked products were evaluated by a panel consisting of 10 members using a scoring system with maximum of 50 scores and a limit of acceptability of 30 scores.

2. Results and discussion

A comparison between some chemical constituents of maize (Balady variety) and fenugreek seeds (Giza 2 variety) is shown in Table 2. Lipids content of fenugreek seeds was similar, but ash content was lower than those generally reported. Protein content of fenugreek seeds was found to be 26.37%. Such value falls within the range reported in the literature (EL-MADFA, 1975). HUSSEIN and NOAMAN (1969) determined higher value (30.2%), while EL-MAHDY and EL-SEBAI (1982) found lower percentage (24%) for Egyptian fenugreek protein content.

Percentage of protein in fenugreek seeds was 2.8 times that of maize grains. Also, ash and lipids contents were markedly higher in fenugreek seds,

Table 2

Proximate chemical composition of maize and fenugreek seeds

Measurement	Maize	Fenugreek
Moisture (%)	8.95	9.03
Lipids[a] (%)	4.36	6.15
Ash[a] (%)	1.40	3.50
Crude protein[a] (%)	9.28	26.37
Digestibility[b] (%)	79.80	32.00

[a] Calculated as percent of dry matter.
[b] Calculated as percent of total protein.

while no considerable difference was found between moisture content of maize and fenugreek.

Digestibility of maize and fenugreek proteins was estimated to be 79.80 and 32.00%, respectively. The markedly low fenugreek digestibility may be attributed to the presence of a trypsin inhibitor (EL-MAHDY & EL-SEBAIY, 1982) and other antinutritive components. It seems likely that the presence of the alkaloid substances which cause the bitter taste considerably impairs the digestibility of fenugreek proteins.

Amino acids profiles of maize and fenugreek seeds are illustrated in Table 3. No considerable differences were observed regarding total amounts of essential amino acids and non-essential amino acids in both maize and fenugreek proteins. Distribution of amino acids in both of the proteins was quite different. Maize protein contained higher amounts of leucine, sulfur amino acids (methionine + cystine), valine, alanine, glutamic acid, histidine and proline, while fenugreek seeds protein was superior in isoleucine, lysine, threonine, arginine, aspartic acid, glycine and serine concentrations. Maize protein is characterized by the high leucine, glutamic acid and proline content as well as low lysine content. On the other hand, fenugreek seeds protein can be characterized by the high arginine, lysine and low methionine + cystine content. These results coincide with those of JAMALIAN and PELLETT (1968).

Table 3

Amino acids composition of maize and fenugreek flours as compared with FAO pattern (g per 100 g protein)

Amino acids	Samples		FAO/WHO pattern (1973)
	Maize	Fenugreek	
Essential amino acids:			
Isoleucine	2.48	4.41	4.00
Leucine	12.18	6.36	7.00
Lysine	3.06	6.06	5.50
Methionine + cystine	3.08	2.28	3.50
Phenylalanine + tyrosine	7.96	7.48	6.00
Threonine	2.99	3.98	4.00
Tryptophan	1.30	1.64	1.00
Valine	4.01	3.27	5.00
Total	37.06	35.48	36.00
Non-essential amino acids:			
Alanine	7.98	3.74	
Arginine	5.31	9.23	
Aspartic acid	6.38	11.84	
Glutamic acid	18.01	15.55	
Glycine	3.59	4.33	
Histidine	3.93	2.81	
Proline	9.54	5.14	
Serine	3.87	4.86	

Essential amino acids of the investigated two proteins compare well with FAO/WHO reference pattern (1973), but the distribution of fenugreek protein essential amino acids appears to be more close to the recommended FAO/WHO pattern.

The data obtained about amino acids composition of maize and fenugreek as well as the digestibility of their proteins were used for the calculation

Table 4

In vitro biological values of maize and fenugreek proteins

Chemical indexes	Samples	
	Maize	Fenugreek
Mitchell index	43.71	40.00
FAO/WHO index	54.25	65.31
Limiting amino acids	Lysine	Methionine + Cystine
Mørup-Olesen index	67.15	88.36

of in vitro biological values by three different chemical indexes. The resulting chemical scores are presented in Table 4. Mitchell index was calculated using essential amino acids of egg protein as a reference. Slight difference was found between maize and fenugreek chemical scores.

FAO/WHO index is also based on the comparison between essential amino acids of the sample and those of egg protein, but the method of calculation is different from that of Mitchell index. The chemical score of fenugreek protein was found to surpass that of maize, and lysine was the limiting amino acid in maize protein, whereas sulfur amino acids (methionine + cystine) were the limiting ones in fenugreek protein.

The method followed for the calculation of Mørup-Olesen index based on essential amino acids composition and also biological experimental data. That is why chemical scores obtained by this method are more reliable. When this index was used, the score of fenugreek protein proved to be much higher than that of maize protein.

In light of the results concerning proximate chemical composition, protein digestibility, amino acids composition and chemical scores of maize and fenugreek, it could be concluded that fenugreek flour, despite of its low protein digestibility, may be used for supplementation of maize flour owing to its high protein and mineral contents, favourable essential amino acids distribution and good chemical score.

In the subsequent part of our work, mixtures of fenugreek and maize flours were used in making bread, salty wafer and sponge cake. Baked products were nutritionally and organoleptically evaluated.

The protein contents of the baked products are illustrated in Table 5. All the obtained values are clearly higher than that of maize flour. Protein content of baked bread progressively increased with the increase of added fenugreek levels. The increase in protein percentage may be also due to, though to a smaller extent, the added yeast and to the loss of non-protein compounds during fermentation and baking process.

Table 5

Protein content of bread, salty wafer and sponge cake made from maize flour supplemented with fenugreek flour

Product	Percent of fenugreek in flour mixture	Protein content %
Bread	5	10.80
	10	11.99
	20	13.45
Salty wafer	5	10.60
	10	11.64
Sponge cake	8	15.45

Percentages are calculated on dry weight basis.

The increase of protein percentage of baked wafer is mainly due to the presence of fenugreek flour. Protein percentage of baked cake was considerably higher than that of maize flour owing to the addition of protein rich sources, i.e. eggs and fenugreek flour.

The amino acid profiles of the various types of baked products are listed in Table 6. Comparing with the amino acid profiles of maize and fenugreek flours (Table 3) it is quite evident that the distribution of amino acids has been greatly changed upon baking. Total percentages of essential amino acids became markedly lower in the baked products. On the other hand, equivalent increases occurred in total values of non-essential amino acids. The decrease in total essential amino acids percentage was less pronounced in case of sponge cake owing to its egg protein content which contains high percentage (49%) of essential amino acids (FEVOLD, 1951).

As a result of chemical changes that took place on baking of bread and salty wafer, losses in most amino acids, namely, tryptophan, sulfur amino acids, aromatic amino acids, isoleucine, leucine, lysine, alanine, histidine and arginine occurred in the order given. On the other hand, the relative amounts of all other investigated amino acis in 100 g protein were increased to different extents. The most heat resistant amino acids were glutamic acid, threonine, valine and aspartic acid in a descending order. These results may be explained by the fact that when proteins are heated with carbohydrates, interaction

Table 6

Amino acids composition of bread, salty wafer and sponge cake made from maize flour supplemented by fenugreek (g per 100 g protein)

Amino acids	Bread			Salty wafer		Sponge cake
	5% FF	10% FF	20% FF	5% FF	10% FF	8% FF
Essential amino acids						
Isoleucine	1.58	1.46	1.49	1.29	1.73	1.53
Leucine	8.05	7.94	6.92	8.26	7.76	5.43
Lysine	2.30	2.39	2.41	2.17	2.50	5.79
Methionine + Cystine	1.70	1.43	1.07	1.71	1.29	1.84
Phenylalanine + Tyrosine	3.53	4.25	4.29	3.80	4.05	5.11
Threonine	4.88	4.38	4.79	4.65	4.29	5.79
Tryptophan	0.59	0.49	0.52	0.41	0.48	1.03
Valine	5.11	5.01	4.86	6.71	5.55	7.32
Total	27.74	27.35	26.35	29.00	27.65	33.84
Non-essential amino acids						
Alanine	4.52	5.09	5.30	5.11	5.04	4.90
Arginine	4.35	4.42	4.67	3.87	4.99	4.49
Aspartic acid	7.63	9.37	11.95	8.73	10.88	11.22
Glutamic acid	33.39	30.10	30.93	30.69	32.55	24.69
Glycine	4.00	4.07	4.79	3.93	4.88	3.95
Histidine	2.85	2.65	2.29	2.69	2.31	2.38
Proline	9.87	9.67	9.40	10.13	10.19	7.14
Serine	5.70	5.15	5.96	5.06	5.41	8.08

FF: Fenugreek flour.

Table 7

In vitro biological values and digestibility of baked products made from maize and fenugreek flour mixtures

Chemical indexes	Bread			Salty wafer		Sponge cake
	5% FF	10% FC	20% FF	5% FF	10% FF	8% FF
Mitchell index	29.23	25.09	18.77	23.89	22.63	28.33
FAO/WHO index	51.31	46.73	41.44	40.02	48.06	40.73
Limiting amino acids	Ile	Ile	Met + Cys	Ile	Met + Cys	Ile
Mørup-Olesen index	54.25	46.22	40.25	39.50	46.29	66.03
Digestibility	78.00	78.50	75.20	79.00	79.50	80.20

FF: Fenugreek flour; Met.: Methionine; Ile: Isoleucine; Cys: Cystine.

of the protein with the carbohydrates occurs, and in consequence of continued heating, browning and destruction of some amino acids take place.

The situation with sponge cake was different, since reasonable percentage of most essential amino acids especially lysine, aromatic amino acids, threonine, tryptophan and valine were still present after baking. Such results may be

Table 8

Organoleptic evaluation of baked products made from maize–fenugreek flour mixtures

Qualities	Bread			Salty wafer[a]		Sponge cake
	5% FF	10% FF	20% FF	5% FF	10% FF	8% FF
Eating quality (10)	7.5	6.5	3.5	8.5	5.5	8.5
Odour (10)	7.0	6.0	5.0	7.5	6.0	8.0
Texture (10)	6.5	5.5	4.0	9.0	7.5	9.5
Crust colour (10)	6.0	5.0	4.5	8.0	7.0	8.5
Crumb colour (10)	5.0	3.0	2.5	—	—	9.0
Total score (10)	32.0	26.0	19.5	33.0	26.0	43.5

[a] Total score = 40; FF: Fenugreek flour.

due to the high initial contents of essential amino acids in cake before baking because of the considerable egg protein content.

The estimated in vitro biological values of the baked products are tabulated in Table 7. Baking resulted in considerable losses of the chemical scores calculated by the different methods as compared with the data in Table 4. The losses in biological values varied with the kind of baked product owing to the different times and temperatures of baking.

The differences in the thermostability of amino acids have led to changing the limiting amino acid in four baked products to become the isoleucine, while methionine + cystine were the limiting in the other two products. Best biological values among baked products were attained in bread containing 5% fenugreek and in sponge cake which contained 8%.

The organoleptic qualities of maize-fenugreek baked products were scored as shown in Table 8. Eating qualities and odour of sponge cake were highly acceptable. Bread and salty wafer containing 5% fenugreek flour were also acceptable, while increasing fenugreek percent in the flour mixtures to reach 10% or 20% led to deterioration of eating qualities and enhanced the unpleasant odour and bitter taste. The sponge cake made from maize–fenugreek flour mixture had an excellent spongy structure and fine texture. Also, salty wafer had good texture, but loaves of bread had coarse structure and thick cell walls; and crumb texture was coarse and crumbly. Colour of crust and crumb of sponge cake containing fenugreek flour was highly accepted, whereas salty wafer and bread had less acceptable colour. The yellow colour caused by addition of fenugreek was darkened with the higher fenugreek percentages.

It can be concluded that introducing 8% fenugreek flour in maize–fenugreek flour mixture to make sponge cake is well accepted. Also 5% fenugreek flour can be used in making acceptable salty wafer and maize–fenugreek bread.

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GRAVIMETRIC DETERMINATION OF THE PHENOLIC FRACTION IN THE LIQUID SMOKE PREPARATIONS UTP-1 AND ITS IDENTIFICATION BY GAS CHROMATOGRAPHY – MASS SPECTROMETRY

P. Šimko, J. Leško, J. Dubravický and M. Lapár

Department of Chemistry and Technology of Saccharides and Foods, Faculty of Chemical Technology, Slovak Technical University, Radlinského 9, 812 37 Bratislava. CSFR

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The paper refers to the quantitative determination of the phenolic fraction which is present in the liquid smoke preparations UTP-1 as well as to identification of the individual compounds in this fraction using gas chromatography and mass spectrometry. The results have shown that the liquid smoke preparations UTP-1 represent virtually 1% solution of phenols, mostly guaiacol, syringol and their derivatives. These compounds participate to a significant extent in the creation of the smoke flavour and their quantities directly determine their suitability and/or application in the process of flavouring of various food items.

Keywords: gas chromatography, liquid smoke preparations, phenols, mass spectrometry

Flavouring of food articles using products of thermal destruction of wood is an important part of the technological process aimed at the creation of a specific organoleptic profile. This one is subsequently participating in the fact that smoked food articles are particularly demanded on the market, with respect to their specific sensory properties.

Flavouring of meat products had been carried out by the use of virtually identical methods applied over centuries, viz. by subjecting them to the effects of thermal destruction products of wood materials, resulting in the preservation in addition to flavouring thereof. The smoke, with its composition depending on several factors, e.g. chemical composition of the wood, access of oxygen during the smoking process to the wood, temperature of smoke generation, moisture contents of the wood, represents a considerably complex mixture of various chemical compounds. So far, more than four hundred volatile compounds have been identified and classified into the following chemical groups: phenols — 75, carbonyls — 131, organic acids — 48, alcohols — 22, esters — 22, furans — 46, lactones — 16, and 50 miscellaneous compounds (Maga, 1987). The dominant influence of phenols on the creation of the smoke flavour was discussed in several papers (Lustre & Issenberg, 1970; Kornreich & Issenberg, 1972; Knowles et al., 1975; Baltes & Sochtig, 1979). However, the formation of the complex organoleptic profile

depends also on further groups, e.g. carbonyls and organic acids. On the other hand, the groups of lactones and pyrazines which also exert a significant influence on the total flavour of smoke have been less studied (MAGA, 1987).

Liquid smoke preparations (LSP) represent a new generation of additives utilizable for purposes of foods flavouring in order to create the characteristic odour and taste of smoked products. Application of these flavour additives offers several advantages as compared to the traditional smoking, e.g. simple handling and thus acceleration of the technological process of production; virtually none of the environmental hazards present in the traditional smoking process — where a mere 5 to 15% of the wood is being used effectively — the rest representing a direct pollution source; possibility of accurate measurement of the sensorily effective components; elimination of harmful compounds (polycyclic aromatic hydrocarbons) in the production process of the LSP as well as the possibility of their rapid determination in the LSP.

Several references have discussed the issue of determination of the phenols in LSP used for smoke flavouring or smoked meat products (SUMAROKOV, 1953; LUSTRE & ISSENBERG, 1970; KISHIMOTO & HIRANO, 1976; WITTKOWSKI et al., 1981; MCGILL, 1985).

This work has been aimed at elaboration of a method for determination of the total amount of the phenolic fraction, and a method for its qualitative determination in the LSP UTP-1.

This new additive is made of a raw wood tar after its homogenization, neutralization and alkalization. Consequently, the wood tar is refined with cyclohexane (removing of polycyclic aromatic hydrocarbons), acidified, filtered and clarified. This way made LSP UTP-1 (CS Patent No. 264 158/89) was tested by Ames's method of gene mutations as well as hygienic-toxicological tests. On the bases of obtained results, the LSP UTP-1 has come into use. At present, however, the tests of chronical toxicity are being carried out at the Institute of Preventive Medicine.

1. Materials and methods

Samples of the LSP UTP-1 were obtained from the manufacturer — Slovenské Lučobné Závody, Hnúšta, CSFR.

Sumarokov's method modified as shown below was used for the quantitative determination of the phenolic fraction:

Four g of the sample were neutralised to pH = 7.5 with saturated $NaHCO_3$ solution, and the neutral sample shaken in a separatory funnel with 3×25 cm^3 of diethyl ether. The etheric layers were alkalized using small volumes of 10% NaOH solution (w/w) until a colourless aqueous layer was obtained. After separation, the aqueous solution of phenolates was then acidified to

pH = 5.2 with 32% H_2SO_4, and the phenols thus released extracting with diethyl ether. Then the etheric layer was dried by passing through a layer of anhydrous Na_2SO_4. The diethyl ether was evaporated in a rotary vacuum evaporator and the remaining traces of solvent were removed by a nitrogen stream. The phenolic fraction was weighed and subsequently used for qualitative analysis by gas chromatography and mass spectrometry.

1.1. Gas chromatography conditions

The chromatograph used was Chrom 5 with a glass column of 2.5 m length, ID = 3 mm. The column was packed with stationary phase Carbowax 20 M (15% (w/w) on Chromosorb W-HMDS 80–100 mesh). Nitrogen was used as carrier gas, at a pressure of 1.2×10^4 Pa. The injection space was heated to 250 °C, and the following temperature program applied: 146 °C isotherm for 6 minutes followed by the gradient 1 °C min^{-1} up to 206 °C; 10 min isotherm. A flame ionization detector (FID) was used for detection of the eluated phenols. Five μl of the phenolic fraction were used for analysis after being isolated as shown above and diluted with diethyl ether (1 : 1). The phenolic compounds were identified adding etheric solution of standards. The standards (guaiacol and the others) were obtained from Merck, Supelco and Lachema.

1.1.2. Gas chromatography — mass spectrometry conditions

A MAT 111 (Varian) was used for identification and confirmation of results of phenols. The chromatographic column (2 m length, ID = 2 mm)

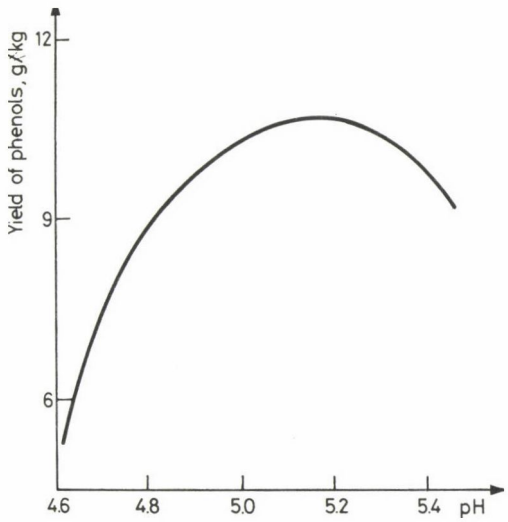

Fig. 1. Gravimetric determination of the phenolic fraction in the LSP UTP-1 and its identification by gas-chromatography – mass spectrometry

filled with the same stationary phase and Chromosorb as mentioned above was made of stainless steel.

Helium was used as carrier gas for analysis. The injector space was heated to 220 °C and helium separator to 200 °C. Ionization current: 270 A, ionic source temperature 200 °C, energy of ionizing electrons at scanning of the mass spectra 80 eV. The following temperature program was applied: the gradient 8 °C min^{-1} from 100 °C to 160 °C.

2. Results

Experimental quantitative determination of the phenolic fraction has shown that the limiting factor influencing the overall yield of the analysis is the grade of acidifying of phenolates. As shown in Fig. 1, the pH value of the solution has affected the yield significantly, with pH = 5.2 found as

Table 1

The results of gravimetric determination of phenolic fractions in the liquid smoke preparations UTP-1

Liquid smoke preparation UTP-1	Concentration of phenols (g per kg)	Standard deviation (%)	Number of replicates
A	10.67	4.9	3
B	11.20	5.4	3
C	9.85	5.2	3

Table 2

Phenols in the liquid smoke preparations UTP-1 and their relative amount

Compound	No. of peak	Liquid smoke preparation UTP-1		
		A (%)	B (%)	C (%)
2,4-dimethyl-1,3-cyclopentadione	1	1.4	1.6	1.5
2-hydroxy-3-methyl-2-cyclopentadione	2	16.9	13.6	14.1
Guaiacol	3	5.9	9.8	12.1
Isomer of dimethylcyclopentadione	4	4.2	5.7	4.3
4-methylguaiacol	5	8.7	7.6	1.1
4-ethylguaiacol	6	9.5	12.1	19.2
Dimethylguaiacol	7	2.3	2.1	0.2
4-propylguaiacol	8	7.0	8.1	6.4
3,4-dimethylphenol	9	0.8	1.3	0.5
2,4-dimethylphenol	10	2.0	3.8	0.9
Eugenol	11	2.0	2.9	0.2
Syringol	12	27.3	19.5	33.6
4-methylsysingol	13	7.4	7.5	6.0
4-ethylsysingol	14	4.5	4.5	0.6
Unknown	X	0.2	0.2	0.1

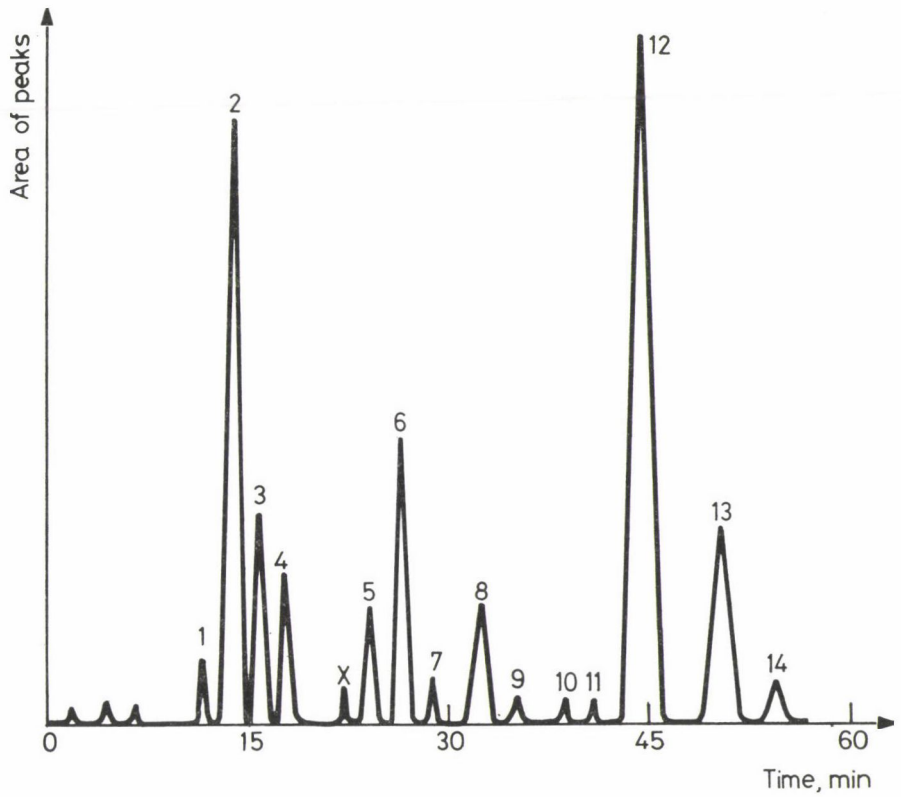

Fig. 2. Gravimetric determination of the phenolic fraction in the LSP UTP-1 and GC-MS

optimum acidity. In all, three samples of LSP UTP-1 were analysed in the course of our experiments, with the isolated fraction weights as shown in Table 1.

By quantitative analysis of the phenolic fraction, 14 compounds were determined, with their relative respective representation, which is shown in Table 2. Figure 2 shows a typical chromatographic record of the isolated phenolic fraction; in this case the disturbing influence of the stationary phase was eliminated by scanning differential signals obtained from parallelly arranged chromatographic columns, i.e. the chromatographic array was operating in the Dual mode.

3. Conclusions

Based on the results we have found that the liquid smoke preparations UTP-1 contain in practice, 1% solutions of phenolic compounds, contributing significantly to the creation of the required smoke flavour. By qualitative

analysis of the phenolic fraction it was found that this fraction consisted of the compounds mainly represented by guaiacol, syringol and their derivatives, described in references as typical compounds, which arise during thermal destruction of wood.

The quantitative determination and identification of the phenolic fraction in liquid smoke preparations is important from the viewpoint of measuring smoke flavour additives into food products since there is a close correlative dependence between the quantity of such additives, and the intensity of the sensory response.

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NEW PURIFIED PLANT PROTEINASES FOR THE FOOD INDUSTRY

N. S. Priolo, L. M. I. Lopez, M. C. Arribére, C. L. Natalucci and N. O. Caffini

Laboratorio de Botánica Aplicada, Facultad de Ciencias Exactas, Universidad Nacional de la Plata, Casilla de Correro 711, 1900 La Plata. Argentina

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A crude enzyme preparation obtained from unripe fruits of *Bromelia hieronymi* Mez (Bromeliaceae) shows a broad pH range of optimum proteolytic activity (more than 90 % of maximum activity between pH 7.2 and 10.0) and a good thermal stability up to 45 °C. Diafiltration followed by ion-exchange chromatography allows the separation of four proteolytic components of closely related molecular weights (26.1–28.6 kD).

Keywords: *Bromelia hieronymi* (Bromeliaceae), cysteine proteinases, enzyme purification, plant proteases

Proteases represent more than a half of the total amount of commercial industrial enzymes presently available on the world market (Mantell et al., 1985). Plant proteases play an important role in food technology and many food industries make use of them in some step of food production (Caffini et al., 1988): tenderization of meat, brewing industry, cheese elaboration, bread manufacture and obtention of modified proteins (enzyme modified cheeses, solubility of soybean, cotton, peanut and wheet proteins, etc.).

The bulk of these industrial enzymes is obtained from microbial sources, but some plant cysteine proteinases, namely bromelain, papain and ficin are still preferred in a number of processes. Bromelain has been obtained from stems (Heinicke & Gortner, 1957) and fruits (Ota et al., 1964) of "pineapple" (*Ananas comosus* L. Merrill, Bromeliaceae) and the proteolytic components of both "stem bromelain" and "fruit bromelain" have been extensively studied (Murachi, 1970, 1976; Ota et al., 1985). This paper deals with the isolation, purification and partial characterization of four proteases from unripe fruits of *Bromelia hieronymi* Mez (Bromeliaceae), a species taxonomically close to pineapple.

1. Materials and methods

Unripe fruits of *Bromelia hieronymi* Mez were collected by the late Mr. Pablo Legname (Fundacion Miguel A. Lillo) in Tucumán Province, Argentina, and stored at −20 °C until the beginning of the extraction procedure. Fruits

(100 g) were chopped and then homogenized for 1 min in a Waring blender with cold (−20 °C) acetone (250 cm^3) in order to obtain an acetone powder. Two g of this powder were extracted for 60 min with 100 cm^3 of 0.1 mol sodium phosphate buffer (pH 7.0) containing 5 mM EDTA and 5 mmol cysteine, with gentle stirring. The suspension was centrifuged at 16,000 × g for 30 min and the precipitate was discarded. The pale yellow supernatant is the crude preparation. All operations were carried out at 0–4 °C.

Proteins were measured according to BRADFORD (1976), using bovine albumin as standard. In all chromatographic procedures protein concentration was estimated by measurement of the absorbance at 280 nm. Carbohydrate content was determined using the method of DUBOIS and co-workers (1956).

For all enzyme activity determinations, 0.1 cm^3 of enzyme solution and 1.1 cm^3 of 1% (w/v) casein dissolved in 0.1 mol sodium phosphate buffer (pH 7.4) containing 12.5 mmol cysteine were incubated at 37 °C for 5 min. The reaction was stopped by addition of 1.8 cm^3 of 5% (w/v) trichloroacetic acid. The tubes were centrifuged at 4000 × g for 20 min and the absorbance of supernatants was measured at 280 nm. An arbitrary enzyme unit (U_{cas}) was defined as the amount of enzyme that produces an increase of one absorbance unit (owing to casein digestion products soluble in 3% (w/v) trichloroacetic acid) per minute at 37 °C and pH 7.4.

Enzyme activity in dependence of pH was measured within the range from pH 6.0 to 11.0. The following buffer solutions were used: 0.1 mol sodium phosphate (pH 6.0–7.5); 0.25 mol sodium borate, containing 0.25 mol potassium chloride (pH 7.5–9.5); 0.25 mol sodium glycinate, containing 0.25 mol sodium chloride (pH 9.5–11.0).

Thermal stability of crude preparations was determined by keeping enzyme solutions for 5, 10, 20, 40, 60, 90 and 120 min at 37 °C, 45 °C, 55 °C and 65 °C respectively, and then measuring the residual activity as indicated above.

Gel chromatography was performed on a column (1.5 × 31 cm) of Sephadex G-75 Superfine equilibrated and eluted with 0.1 mol sodium phosphate buffer (pH 7.0).

As a first purification step 10 cm^3 of crude preparation was defiltrated (Amicon 8050, membrane YM 10) with 100 cm^3 of 50 mmol Tris-HCl buffer (pH 8.0), and the final volume (10 cm^3) applied to a column (1.5 × 30 cm) of DEAE-Sepharose CL-68 equilibrated with 50 mmol Tris-HCl buffer (pH 8.0). After washing the column with 70 cm^3 of the same buffer, the retained proteins were eluted with 200 cm^3 of a 0.2–0.6 mol sodium chloride linear gradient in the starting buffer. The unretained fraction was then applied to a column (1.5 × 30 cm) of CM-Sepharose CL-6B equilibrated with 50 mmol Tris-HCl buffer (pH 8.0). After washing the column with 65 cm^3 of the same buffer,

the elution was performed with 200 cm^3 of a 0.0–1.0 mol sodium chloride linear gradient in the starting buffer.

SDS-Polyacrylamide gel electrophoresis was performed following the method of LAEMMLI (1970). Current was kept constant at 20 mA during stacking and then increased to 30 mA and kept constant for 3 h. Staining procedure with Coomassie Brillant Blue R-250 was done as described by HAMES (1981).

2. Results and discussion

2.1. Crude preparations

As in the case of other Bromeliaceae (BOLLER, 1986), fruits of *Bromelia hieronymi* Mez contain cystein proteinases (p-hydroxymercuribenzoate and mercuric chloride inhibit the enzyme in a reversible way and cysteine acts as activator) that can be extracted from acetone powder with neutral buffers containing protective substances. Best results are obtained when EDTA and cysteine (5 mmol final concentration) are added to the buffer.

Crude preparations exhibit a broad range of pH within activity is notably high (over 90% between pH 7.2 and 10.0), property that makes them specially valuable in processes that take place in neutral-weak alkaline media (Fig. 1). Thermal stability of the crude enzyme is not a remarkable feature (Fig. 2): activity is not affected after 2 h at 37 °C and even is fairly high at 45 °C (75% of residual activity after 2 h), but higher temperatures cause significant inactivation (only 35% of activity is retained after 2 h at 55 °C). Thermal behaviour of the crude enzyme is somehow a useful property, since it could be easily inactivated in the foodstuffs, and so the active enzyme does not get into the organism. Unlike stem bromelain (MURACHI et al., 1964),

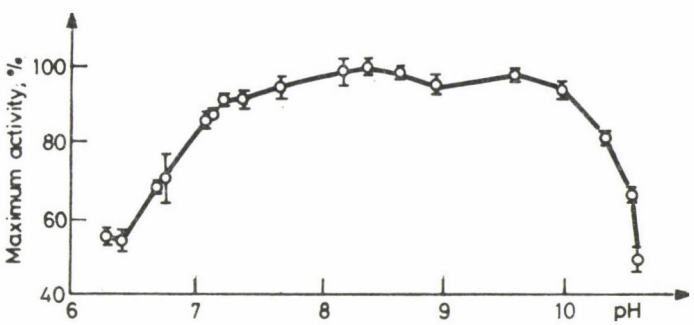

Fig. 1. Effect of pH on the activity of crude preparations. Proteolytic activity in dependence of pH was measured using 1% (w/v) casein as substrate. Values consigned correspond to pH of assay mixtures. Data points represent the mean value of five determinations and each experiment was repeated twice. Vertical lines indicate standard deviations

lyophyllization seems to be an appropriate way for keeping crude preparations, as enzyme activity is only slightly reduced (8.6%) when freeze-dried preparations are redissolved.

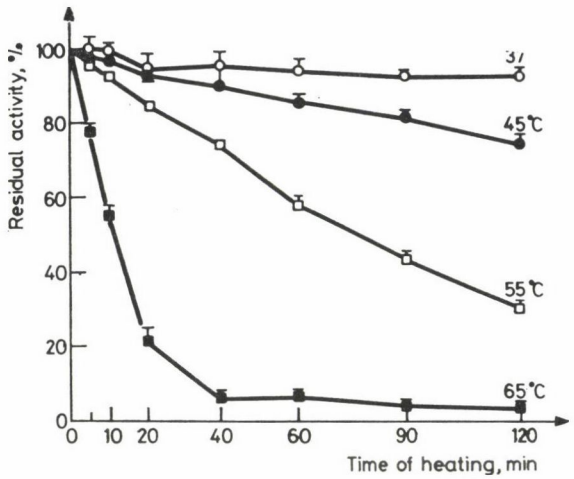

Fig. 2. Effect of different heat treatments on the stability of crude preparations. Data points represent the mean value of five determinations and each experiment was repeated twice. I: standard deviation

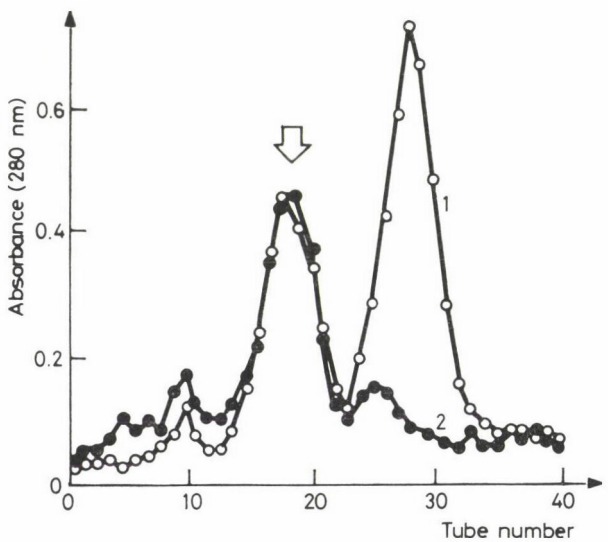

Fig. 3. Evidence for elimination of low molecular weight inactive compounds from crude preparations by ultrafiltration. Elution pattern (absorbance at 280 nm) of gel filtration on Sephadex G-75 Superfine. 1: crude preparation; 2: ultrafiltrate. The arrow indicates the proteolytic fraction

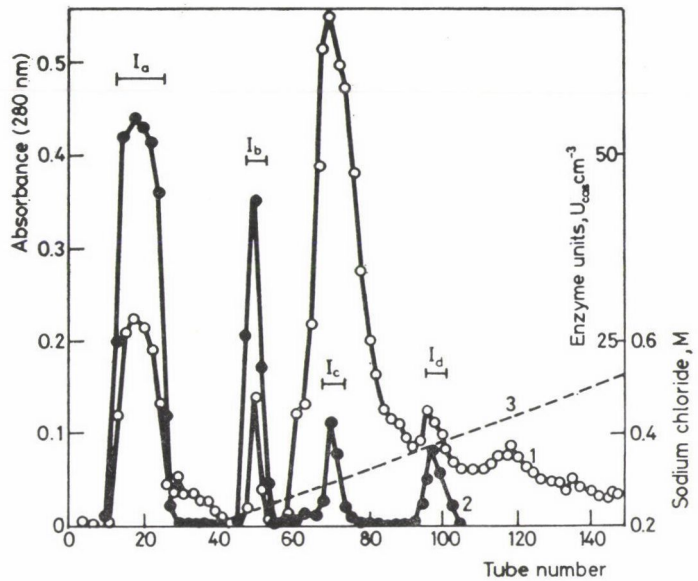

Fig. 4. Chromatography on DEAE-Sepharose CL-6B of the ultrafiltrate. Starting buffer: 50 mM Tris-HCl (pH 8.0); flow rate: 9.6 cm^3 h^{-1}; fractions of 1.6 cm^3 were collected 1: absorbance at 280 nm; 2: caseinolytic activity; 3: sodium chloride gradient. Bars indicate the fractions that were pooled

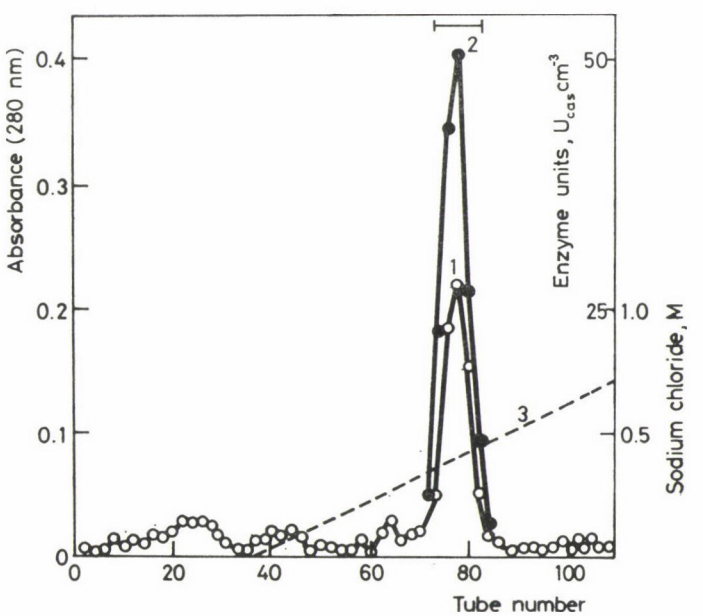

Fig. 5. Chromatography on CM-Sepharose CL-6B of the unretained fraction (I_a). Starting buffer: 50 mmol Tris-HCl (pH 8.0); flow rate: 9.6 cm^3 h^{-1}; fractions of 1.6 cm^3 were collected 1: absorbance at 280 nm; 2: caseinolytic activity; 3: sodium chloride gradient. Bars indicate the fractions that were pooled

2.2. *Purification*

Crude preparations contain 1.4. mg of soluble protein per g of fresh fruits and a relatively high carbohydrate content (carbohydrate–protein ratio = 5.0). Diafiltration reduces carbohydrate–protein ratio to one third and eliminates the bulk of low molecular weight inactive compounds, as evidenced by gel filtration (Fig. 3).

The main proteolytic (basic) fraction (I_a) elutes with the starting buffer (50 mmol Tris-HCl, pH 8.0) when anion exchange chromatography on DEAE-Sepharose CL-6B is performed (Fig. 4), while another three fractions (I_b, I_c and I_d) leave the column only when a sodium chloride linear gradient is applied (0.24 mol, 0.32 mol and 0.39 mol, respectively). When I_a is pooled and applied to a CM-Sepharose CL-6B column (Fig. 5), a single, more purified (6.9 fold) fraction is obtained when the sodium chloride linear gradient reaches 0.4 mol concentration (fraction II). The purification scheme is given in Table 1.

SDS-polyacrylamide gel electrophoresis shows (Fig. 6) that relative molecular weights of the purified proteolytic fractions are closely related (between 26 100 and 28 600). These values are of the same order as those presented by OTA and co-workers (1985) and TAKAHASHI and co-workers (1973) for the

Table 1

Purification of four proteases from unripe fruits of Bromelia hieronymi *Mez* (*Bromeliaceae*)
All enzyme assay were carried out at 37 °C, pH 7.4, with 1% casein (w/v) containing 12.5 mmol cysteine as substrate

Step	Volume (cm^3)	Protein ($mg\ cm^{-3}$)	Total protein (mg)	Activity ($U_{cas}\ cm^{-3}$)	Total activity (U_{cas})
Crude preparation	10.0	0.873	8.730	208.8	2088.0
Diafiltrate	10.0	0.729	7.290	195.6	1956.0
I_a	20.8	0.032	0.666	48.3	1004.6
I_b	9.6	0.009	0.086	22.9	219.8
I_c	9.6	0.077	0.739	9.3	89.3
I_d	9.6	0.018	0.173	4.9	47.0
II	16.0	0.021	0.336	34.9	558.4

Step	Specific activity ($U_{cas}\ mg^{-1}$ protein)	Purification (-fold)	Yield (%)	Carbohydrate content (mg glucose per cm^3)	Carbohydrate–protein ratio
Crude preparation	239.2	1.0	100.0	4.375	5.01
Diafiltrate	268.3	1.1	93.7	1.250	1.71
I_a	1509.4	6.3	48.1	0.073	2.28
I_b	2555.8	10.7	10.5	0.005	0.56
I_c	120.8	0.5	4.3	0.017	0.22
I_d	271.7	1.1	2.3	0.018	1.00
II	1661.9	6.9	26.7	0.003	0.14

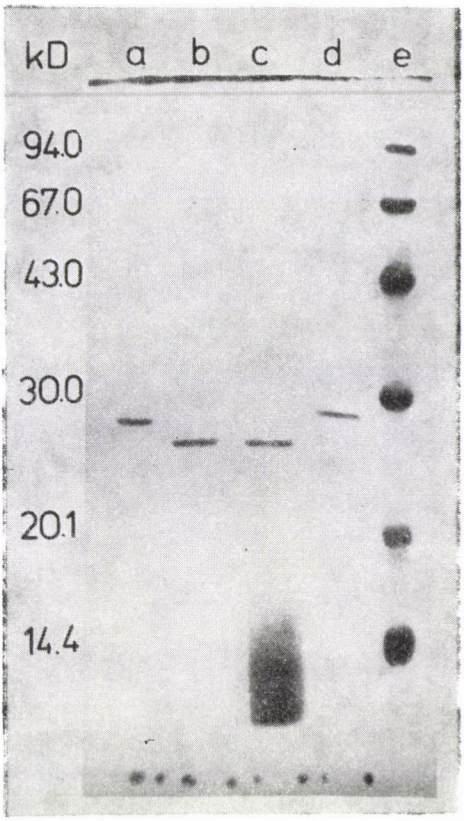

Fig. 6. SDS-Polyacrylamide gel electrophoresis of purified enzymes, according to the methanol of LAEMMLI (1970). 12% polyacrylamide gel was run with: a) fraction II; b) fraction I_d; c) fraction I_c; d) fraction I_b; e) standard protein molecular weight markers (α lactalbumin (M_r 14 400), soybean trypsin inhibitor (M_r 20 100), carbonic anhydrase (M_r 30 000), ovalbumin (M_r 43 000), bovine serum albumin (M_r 67 000), and phosphorylase b (M_r 94 000))

main components of stem bromelain (M_r 27 000 and M_r 25 600, respectively) but are intermediate between those of OTA and co-workers (1985) and YAMADA and co-workers (1976) for the main component of fruit bromelain (M_r 23 000 and 31 000, respectively), all of them determined by the same method. The electrophoretic pattern also confirms the purification degree claimed in Table 1 for fractions I_b, I_d and II; fraction I_c is instead not homogeneous and associated to low molecular weight compounds that can be easily removed by gel filtration.

*

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EFFECT OF OVEN TEMPERATURE VARIATIONS UP ON THE DRYING BEHAVIOUR OF THIN BISCUITS

MAHIR TURHAN and MUSTAFA ÖZILGEN

Food Engineering Department, Middle East Technical University, 06531 Ankara. Turkey

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Numerous physical and chemical changes occur in the products during the baking process. These changes may affect the migration mechanism of water and the drying behaviour of the product. In the present study temperature effects on the drying behaviour of raw thin biscuits of $7 \times 5 \times 0.25$ cm dimensions were studied in a laboratory oven by applying constant or linearly decreasing oven temperatures between 100 and 195 °C. With constant oven temperatures a falling rate drying model, and with linearly decreasing oven temperatures a constant rate drying model stimulated these processes. Final moisture contents are one of the major properties determining the shelf life of the biscuits. These models may be used as simple methods for calculating the baking times.

Keywords: drying models, varying and constant oven temperatures, thin biscuits

During a baking process simultaneous heat and mass transfer take place in the products. In such a process three distinct stages are recognized (SKJÖLDEBRAND & HALLSTRÖM, 1980). The first stage is the preheating of the product surface from the initial temperature to the wet-bulb temperature. The second stage is named in the drying terminology as the constant rate period, where the surface is covered with a continuous water film and retains the wet-bulb temperature. The rate limiting step of the constant rate period is the rate of evaporation on the surface (GEANKOPLIS, 1983). When water transfer rates from the depths of the surface become lower than the surface evaporation rates, the surface water film starts shrinking and exposes the dry product, then the temperature of the dry zones starts increasing over the wet-bulb temperature. This is the third step in the baking process and referred to as falling rate period. In the constant rate period the moisture loss rate is constant:

$$\frac{dX}{dt} = -\Phi \tag{1}$$

where variable X is the fraction of water in the product on dry basis, i.e., weight of water in the product / weight of the dry product. Equation 1 may be integrated as:

$$X = X_0 - \Phi t, \tag{2}$$

The simplest model for moisture transport in the falling rate drying period is expressed by Fick's law:

$$\frac{\delta X}{\delta t} = D \frac{\delta^2 X}{\delta Z^2} \tag{3}$$

When diffusivity D is constant, the initial moisture content of the food is uniform, volume changes of the product are negligible and surface moisture is in equilibrium with water vapor in the oven, solution of Eqn. 3 for an infinite slab is as follows (PORTER et al., 1973; GEANKOPLIS, 1983):

$$\frac{X - X^*}{X_0 - X^*} = \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left[\frac{(2n+1)^2 \pi^2}{4} \frac{Dt}{L^2}\right] \tag{4}$$

Equation 4 may be used for modeling drying of foodstuffs after correcting diffusivity for the volume changes by assuming D/L^2 a constant (VACCAREZZA & CHRIFE, 1975). For long drying times Eqn 4 may be simplified with one term approximation and rearranged:

$$\ln (X - X^*) = \ln [(X_0 - X^*)B] - K t \tag{5}$$

where $B = 8/\pi^2$, $K = \pi^2 D/4 L^2$ and parameter K is referred to as the drying rate constant.

In a baking oven heat effects create various physical and chemical changes in the biscuits. The major chemical changes may include gas formation, denaturation and coagulation of the proteins, gelatinization of starch, crust formation and browning reactions (SMITH, 1966). The major physical changes may include evaporation of water, expansion of the volume, and development of porous or spongy structure. These changes depend on the temperature programming of the oven. The structural changes affect the migration of water in the product thus the drying behaviour depend on the baking temperature variations. In the present study these effects will be studied by means of different drying models. The final moisture content is one of the most important parameters determining the shelf life of the biscuits and it is mostly required to be less than 4%. The simple models presented in this paper may also be used as fast and simple calculation methods of the baking times.

1. Materials and methods

Biscuits were made by baking the dough. The dough was obtained from a local commercial biscuits factory and made of wheat flour, sugar, invert sugar, hydrogenated vegetable oil, vaniline, lecithin, fresh milk, salt and leavening agents. Individual biscuits were of about $7 \times 5 \times 0.25$ cm dimensions

at the beginning of the baking process. Thickness of the biscuits was almost doubled after baking. Pin holes were made through the smallest dimension of the biscuits to help in the baking process. Pin holes were in square array with one cm distance from one an other. Baked biscuits structure was developed during the baking process and finally a porous structure was attained.

Experiments were carried out in a typical laboratory baking oven with no air circulation. Biscuits were baked in two different processes. In the first process the oven temperatures were constant at 100, 125, 150 and 175 °C. In the second process oven temperatures decreased linearly with time as follows:

$$T = T_0 - \alpha t. \tag{6}$$

Biscuits occupied only a very small part of the oven. There were two circular aeration holes of 2 cm diameter on both sides of the oven. These holes were kept open during the experiments, thus vapour accumulation in the oven was prevented. Shelves of the oven had been replaced with a typical baking steel belt (open area 40%). Initial moisture contents of the biscuits were determined before entering into the oven by the help of a moisture analyzer (Brabender, W. Germany type HAV, accuracy $\pm 0.1\%$). Mass was measured on a laboratory balance (Sauter, W. Germany, type 414/10, sensitive to 0.0001 g). Data were taken after 5, 10, 15, 20, 25 and 30 min, respectively, of baking. Each data point was established as the average of six biscuits.

2. Results and discussion

In the baking process more than 100% increase was observed in the thickness of the biscuits. The structural change from dough to the baked biscuits occurred gradually with the associated changes as described earlier. The biscuits attained a porous structure at the end of the baking process.

In a baking process at high temperatures the water film of the surface is evaporated rapidly, therefore the surface of temperature increasses above the wet-bulb temperature and affects the temperature and the moisture profiles in the product. Rates of the chemical reactions, i.e. starch gelatinization, protein coagulation, etc. depends on temperature. Increasing the surface temperature over the wet-bulb temperature may cause formation of a gelatinized layer on the surface, and slow down diffusion of water. There are also reports in the literature implying that diffusion of water strongly depends on the moisture contents of the biscuits (TUNG & LUND, 1990) or gelatinized starch (KARATHANOS et al., 1990). Overheating the surface may decrease the water content of the product near the surface, thus affects the diffusion rates and drying behaviour of the product.

When the biscuits were baked in the constant temperature oven a falling rate period drying model, i.e. Eqn. 5, simulated the moisture loss phenomena implying that the water film on the surface was rapidly evaporated, and the surface attained a higher temperature than the wet-bulb temperature. This phenomena were simulated with Eqn. 5 and when $\ln (X^* - X)$ was plotted against time slope of an individual line was K and its intercept with $t = 0$ axis was $\ln [(X - X^*)B]$ (Fig. 1). Numerical values of parameters K were given in the legends to the figures. Correlation coefficients of these lines were between -0.98 and -1.0 indicating an almost perfect fit. Numerical values of parameter X^* were almost zero in all the experiments. When the biscuits were baked by applying decreasing oven temperature, Eqn. 5 did not simulate the data; but a constant rate drying model, i.e. Eqn. 1, showed an almost perfect fit when the numerical values of variable X were greater than 0.01 (Fig. 2). This result implies that with decreasing oven temperatures the surface water film was present and the surface was maintained at the wet-bulb temperature for a longer period than in the previous case. Numerical values of parameter Φ and variation of the oven temperatures are given in the legend to Fig. 2. It can be seen in both Figs. 1 and 2 that the drying rates were higher with higher temperatures. At the same time numerical values of variable X were generally higher in Fig. 1 when compared to those in Fig. 2 with similar initial temperatures.

In the present study, since the moisture transfer rates were limited by the diffusion rates through the product, no air circulation was employed in

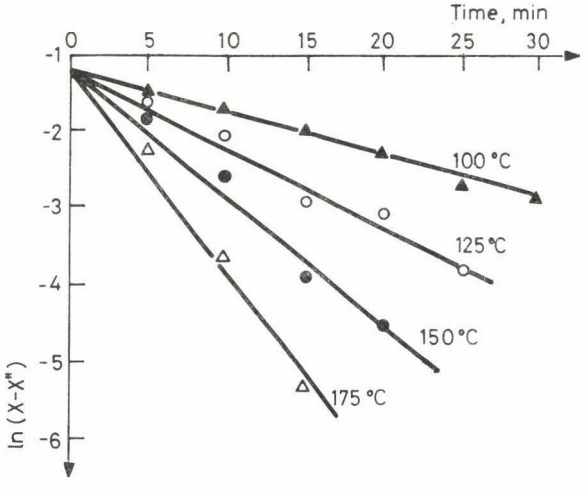

Fig. 1. Comparison of the falling rate drying model with the data (△, ○, ●, △) obtained in the constant oven temperature experiments. The solid lines were the best fitting lines. Experimental data were shown in symbols, numerical values of parameter K were as follows: (▲) 100 °C, $K = 0.9 \times 10^{-3} s^{-1}$; (○) 125 °C, $K = 1.67 \times 10^{-3} s^{-1}$; (●) 150 °C, $K = 2.82 \times 10^{-3} s^{-1}$; (△) 175 °C, $K = 4.48 \times 10^{-3} s^{-1}$

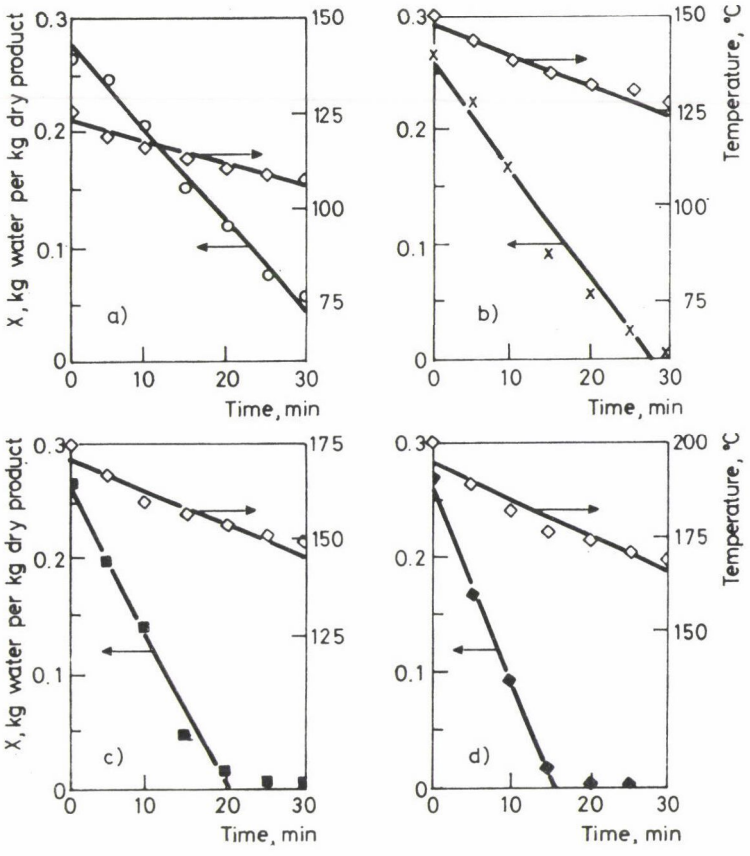

Fig. 2. Variation of oven temperatures and comparison of the constant rate drying model (——) with the experimental data (○, ×, ■, ◆) in the experiments with decreasing oven temperature. Oven temperatures and the constant drying rates in these experiments were:

a) $T = 123 - 0.009t$, $\Phi = 1.25 \times 10^{-4} s^{-1}$
b) $T = 147 - 0.012t$, $\Phi = 1.5 \times 10^{-4} s^{-1}$
c) $T = 171 - 0.013t$, $\Phi = 2.0 \times 10^{-4} s^{-1}$
d) $T = 195 - 0.016t$, $\Phi = 2.7 \times 10^{-4} s^{-1}$

(◇) Oven temperature, (——) temperature model, i.e., Eq. 6. Correlation coefficients of all the data were better than — 0.95

the oven. The air flow rates can affect the external mass transfer rates only and do not have any effect on the diffusion rates. Temperature control on the surface was one of the major goals in the present study. Therefore rapid heat transfer with radiation was not desirable and the electric heating resistances were not directly exposed to the products. Build-up of water in the oven would affect the results by increasing the numerical values of parameter X^* in Eqn. 5. Therefore accumulation of water in the oven was not desired.

Agreement of the models with the experimental data were assessed by standard error of estimate:

$$S = \left(\frac{1}{n} \sum_{i=1}^{n} [X_{\text{exp, i}} - X_{\text{mod, i}}]^2 \right)^{1/2} \quad (7)$$

Typical standard error of estimate associated with the models was about 0.006 kg water per kg dry solids.

3. Conclusions

The chemical and physical structure of the baked product is affected by the temperature programming of the oven. These structural differences affect water migration, therefore different models simulate the drying phenomena. In the baking process with constant oven temperature the falling period drying model, and in the processes where oven temperature decreased linearly with time the constant rate drying model agreed with the data.

Symbols

B: Constant in Eqn 5 (Dimensionless)
D: Diffusivity ($m^2\ s^{-1}$)
K: Drying constant (s^{-1})
L: Half of the distance, through which diffusion occurs (m)
n: Number of data points used in Eqn 7
S: Standard error of estimate associated with variable X (kg/kg)
t: Time (s, min)
T: Temperature (° C)
X: Mass of water per unit dry mass of biscuits (kg/kg)
X^*: Numerical value of X in equilibrium with moisture in the oven (kg/kg)
$X_{\text{exp, i}}$: Experimental value of variable X at the i^{th} data point (kg/kg)
$X_{\text{mod, i}}$: Numerical value of variable X as predicted by the model and corresponding to the i^{th} data point (kg/kg)
X_0: Numerical value of parameter X at the beginning of the baking process (kg/kg)
Z: Distance in the diffusion direction (m)
α: Constant in Eqn 6 (s)
Φ: Constant in Eqn 1 (kg water/kg product s)

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PRODUCTION OF SINGLE CELL PROTEIN FROM YEAST GROWN IN WHEY

FATMA I. EL-HAWARY[a] and AWATIF S. MEHANNA[b]

[a]Botany Department, Faculty of Agriculture, Mansoura University, El Mansoura. Egypt
[b]Dairy Department, Faculty of Agriculture, Tanta University, Kafr El-Sheikh. Egypt

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Single cell protein production by *Kluyveromyces marxianus* in whey under various cultural and nutritional conditions was optimized. Maximum biomass yield having higher crude protein and nucleic acid contents was obtained at pH 4.5 and incubation temperature of 35 °C. Supplementation of sweet and 10% salted whey media with 2% galactose, 0.5% corn steep liquor or 0.5% yeast extract enhanced the cell growth and B-galactosidase production. Ammonium sulfate and lactose had a slight effect on increasing the biomass yield, while ammonium chloride and dipotassium phosphate had no effect. The dilution of 10% salted whey medium stimulated the biomass of *K. marxianus* and had no effect on crude protein and nucleic acid contents.

Keywords: SCP productions, biomass, whey medium

In recognition of the world's wide need for more dietary proteins extensive efforts were made to develop unconventional method for food production. In recent years, microorganisms have attracted a great deal of interest as producers of single cell protein (SCP) using different substrates. This may be due to the high speed in microbial multiplication and the suitability of different substrates for their growth (TANNENBAUM & WANG, 1975).

Whey, which is the main by-production of the cheese industry and discarded in many countries as waste materials, may offer a low cost medium for growth of these microorganisms. On the other hand, cheese whey represents a most troublesome product causing pollution problems because of its biological oxygen demand (BOD). This high BOD is due mainly to lactose which is present in concentrations between 4.5 and 5.0%. Cheese whey contains also relatively high levels of other nutrients such as fats (0.3%), lactic acid (0.2%) and 0.4% nitrogenous materials (GLASS & HEDRICK, 1977; ZALL et al., 1979), that make it suitable as a microbial culture medium (WASSERMAN, 1960; FRIEND & SHAHANI, 1979).

Whey can be used as a medium for yeast growth, since its lactose is considered as a good source for carbon and energy. Yeast grown on whey contained higher proteins and amino nitrogen than those grown on other media (NOUR EL DEIN 1980). Thus, the usage of whey for growth of yeasts may offer a suitable mean for obtaining a low cost enriched feed and food

supplement. Accordingly, the objective of the present work was to isolate yeast strains and to optimize the conditions of biomass production using sweet and salted whey.

1. Materials and methods

Sweet whey was obtained from Misr Milk and Food Company, Sakha Plant, Kafr El-Sheikh, and salted whey was prepared by dissolving NaCl 10%. Enriched cultures were prepared from whey, set up with samples from soft cheese and yoghurt incubated at 35 °C and 2.0 for 5 days. Cultures that gave relatively high growth yield were selected. Pure cultures were obtained after several transplantations on whey agar slants containing 2% agar, 3% malt extract and 0.3% peptone. Stock cultures were stored at 5 °C. The selected isolate was identified as *Kluyveromyces marxianus*.

1.1. Cultivation and harvesting

Sweet whey was deproteinated by adjustment the pH of whey to 3.5 with H_2SO_4(0.1 *N*), then heated to about 97 °C. The whey was cooled and filtrated through cotton to remove the precipitated proteins. Two hundred cm^3 of each kind of whey (sweet and 10% salted) were sterilized in 500 cm^3 Erlenmeyer flasks at 121 °C for 10 min, cooled, inoculated with 1 cm^3 of one week-old culture of *K. marxianus*, and then incubated at 35 °C with agitation in shaking incubator at 200 r.p.m.

The biomass was harvested by centrifugation at 10 000 r.p.m. for 10 min, washed repeatedly with distilled water and recentrifugated under the same conditions. The cells were then ovendried at 60 °C till constant weight. The yield of biomass was evaluated. The growth was determined every hour for 24 h, then every day for 5 days, to obtaine the relation between optical density at 430 nm and dry cell weight as calibration curve and calculated by using regression analysis.

Crude protein content was determined according to Kjeldahl's method (A.O.A.C., 1975). Nucleic acids content was estimated according to the method of SCHNEIDER (1945). The nucleic acid concentration was calculated from standard curve using pure yeast RNA.

The specific growth rate (kh^{-1}) was computed by the following equation:

$$K = \frac{dx}{xdt} = 2.303 \frac{(\log x_2 - \log x_1)}{t_2 - t_1}$$

in which x_1 and x_2 are the absorbances at 430 nm for the cell suspension or dry weight at time t_1 and t_2, respectively (NORRIS & RIBBONS, 1969).

1.2. Effect of temperature

Incubation for a period of 5 days was carried out atthe following temperatures 20, 25, 30, 35 and 40 °C.

1.3. Effect of pH

The whey medium was adjusted to different pH values of 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 using H_2SO_4 (0.1 *N*) and NaOH (0.1 *N*) at optimized temperature for 5 days.

1.4. Effect of medium supplementations

The effects of adding various nitrogen, carbon sources and phosphate salts on biomass yield and protein content were studied by supplementing salted and sweet whey media with 0.5% from each of ammonium sulfate, ammonium chloride, phosphate salt (K_2HPO_4), corn steep liquor and yeast extract. Effect of adding lactose and galactose (2%) was also studied. The starting lactose content of whey medium was 4.5%.

1.5. Quantitative determination of amino acid content

Amino acid analyses were carried out on 30 mg dried yeast samples hydrolyzed with 4 cm^3 6 *N* HCl in closed ampules at 1.05 °C for 24 h. (SPACKMAN et al., 1985). Amino acid determination was carried out in an automatic amino acid analyzer in Food Chemistry Department Technical University Budapest, Hungary.

1.6. β-galactosidase assay

β-galactosidase activity was estimated by measuring the amount of glucose release on hydrolysis of lactose by the cell-free extract according to SZABÓ and DAVIES (1964). GOD-period method (WERNER & WIELINGER, 1970) was applied to follow the release of glucose.

2. Results and discussion

2.1. Effect of pH on cell growth

The results illustrated in Fig. 1, depict the highest biomass yield at pH 4.5 in both media. The maximum biomass yield of *K. marxianus* grown on whey medium decreased more rapidly at higher pH salted whey media

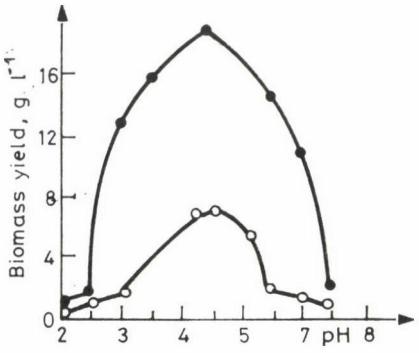

Fig. 1. Effect of pH on *K. marxianus* grown in sweet whey (●——●) and salted whey (○——○) for 5 days

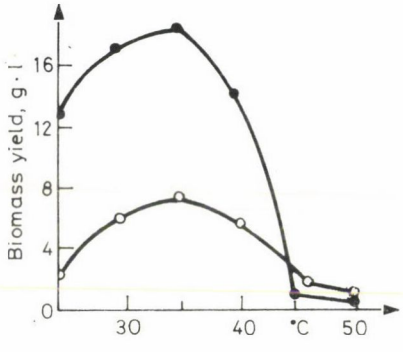

Fig. 2. Effect of incubation temperature on *K. marxianus* grown in sweet whey (●——●) and salted whey (○——○) for 5 days

than sweet whey media. These results are similar to those reported by CASTILLO and SANCHEZ (1978). NOUR EL-DEIN (1980), BARBOSA and co-workers (1984) and GARG and AGRAWAL (1988), while VANANUVAT and KINSELLA (1975) and CHAMPLUVIER and co-workers (1988) reported that the maximum growth of Kluyveromyces spp. were at pH 5.0 and 6.5.

2.2. *Effect of incubation temperature on cell growth*

Results concerning effect of incubation temperature on cell growth are represented in Fig. 2. The maximum biomass yield was obtained at 35 °C for *K. marxianus* grown in both sweet and salted whey. The highest biomass yields were found to be 19.11 (g l^{-1}) dry weight and 7.3 g l^{-1} dry weight for sweet and salted whey, respectively. Biomass yield was found to decrease with increasing incubation temperature higher than 35 °C.

The present results confirmed the conclusion reported by NOUR EL-DEIN (1980) and BARBOSA and co-workers (1984). On the other hand, EL-DIWANY and co-workers (1987) applied 30 °C for Debaryomyces strains and EL-NIMR and co-workers (1982) used 28 °C for optimum cultivation for Kluyveromyces spp. on 6% salted whey medium.

2.3. *Effect of supplementation of the whey medium*

Whey medium was supplemented with $(NH_4)_2SO_4$ (0.5%) and NH_4Cl (0.5%) of equivalent amount as reported by NOUR EL-DEIN (1980).

Specific growth rate was slightly accelerated by addition of $(NH_4)_2SO_4$ (0.5%), while NH_4Cl (0.5%) had no effect on the cell yield. Tables 1 and 2 indicate that wide differences in biomass yield were observed between control and sweet and salted whey media supplemented with different supplementations.

On the other hand, the growth of *K. marxianus* greatly increased on addition of corn steep liquor (0.5%) or yeast extract (0.5%). Stimulation the total cell mass was approximately two folds more than the control (9.11 g l^{-1} dry weight) (Table 1).

Galactose and lactose (2%) increased the total dry weight. The growth rate attained its maximum value when the whey media were supplemented with corn steep liquor or corn steep liquor and galactose or yeast extract and lactose (0.49 h^{-1}, 0.51 h^{-1}, and 0.41 h^{-1}, respectively) in comparison with control (0.21 h^{-1}) as shown in Table 1. Our results corroborate those of NOUR EL-DEIN (1980) and EL-NIMR and co-workers (1982), while differed from those obtained by WENDROFF and co-workers (1970). Table 1 shows

Table 1

Effect of medium composition on specific growth rate, cell yield, crude protein content and nucleic acid content of K. marxianus *grown on sweet whey medium*

Treatment	Specific growth rate (K h^{-1})	Dry cells (g l^{-1})	Crude protein (%)	Nucleic acids (%)
Sweet whey	0.21	9.11	39.40	7.05
Sweet whey + $(NH_4)_2SO_4$	0.31	10.21	45.68	7.90
Sweet whey + NH_4Cl	0.26	7.61	39.46	7.45
Sweet whey + K_2HPO_4	0.28	7.90	42.42	7.50
Sweet whey + yeast extract	0.41	15.51	48.85	8.80
Sweet whey + corn steep liquor	0.49	18.92	49.67	8.91
Sweet whey + lactose	0.29	12.54	44.50	7.11
Sweet whey + galactose	0.39	14.65	48.22	8.99
Sweet whey + yeast extract + $(NH_4)_2SO_4$	0.40	15.90	47.95	8.50
Sweet whey + corn steep liquor + $(NH_4)_2SO_4$	0.46	17.22	48.88	5.59
Sweet whey + corn steep liquor + galactose	0.51	19.11	49.91	9.15
Sweet whey + yeast extract + lactose	0.41	15.85	46.50	8.99
Diluted sweet whey (1:1)	0.15	4.01	34.21	5.90

Table 2

Effect of medium composition on specific growth rate, cell yield crude protein content and nucleic acid content of K. marxianus *grown on salted whey medium*

Treatment	Specific growth rate ($K\ h^{-1}$)	Dry cells ($g\ l^{-1}$)	Crude protein (%)	Nucleic acid (%)
Salted whey 10%	0.17	5.13	29.14	4.33
Salted whey + $(NH_4)_2SO_4$	0.21	6.20	36.41	6.95
Salted whey + NH_4Cl	0.175	4.50	30.10	7.68
Salted whey + K_2HPO_4	0.18	4.40	30.11	7.90
Salted whey + yeast extract	0.29	7.80	37.95	8.90
Salted whey + corn steep liquor	0.31	7.40	38.12	8.95
Salted whey + lactose	0.24	5.98	31.05	7.59
Salted whey + galactose	0.30	6.81	37.55	6.11
Salted whey + yeast extract + $(NH_4)_2SO_4$	0.28	6.66	36.11	5.15
Salted whey + corn steep liquor + $(NH_4)_2SO_4$	0.31	7.40	35.91	6.54
Salted whey + corn steep liquor + galactose	0.33	7.91	38.88	8.88
Salted whey + yeast extract + lactose	0.30	6.24	37.11	6.86
Diluted salted whey (1:1)	0.15	5.82	30.15	4.23

also that the dilution of sweet whey with water (1 : 1 v/v) decreased the biomass yield. This may be due to the dilution the percentage of the available nutrients utilized (VANANUVAT & KINSELLA, 1976). The results presented show that the protein and nucleic acids contents were markedly increased by supplemeting the sweet whey medium with corn steep liquor or yeast extract and with galactose.

The same trend was observed (Tables 1, 2) with respect to cell growth in supplemented media with sweet and salted whey. The dilution of salted whey medium stimulated the biomass yield and had not bad effect on nucleic acid content.

From the foregoing results it could be concluded that the previous supplementations of sweet and salted whey media improved the biomass yield, protein and nucleic acid contents. Maximum biomass yield having higher crude proteins and nucleic acid contents were obtained by cultivation of *K. marxianus* on sweet whey medium at pH 4.5 at incubation temperature of 35 °C with supplementation with galactose or corn steep liquor or yeast extract and lactose.

2.4. Effect of medium composition on the essential amino acid content of K. marxianus

The raw protein content gives a rough idea of the nutrition value of yeast. However, the biological value of protein might show great differences depending on the amounts of essential amino acids for human. As indicated in Table 3, the medium composition affects the concentration of the various amino acids. It is clear that whey medium containing 0.5% corn steep liquor

Table 3

Amino acid composition of protein in biomass of K. marxianus *grown in different media*

Amino acids	1	2	3	4
	(mg amino acid per g sample)			
Lysine	40.12	56.02	58.94	54.44
Threonine	22.65	33.17	38.12	94.89
Valine	23.36	30.49	33.37	51.84
Leucine	26.49	41.40	56.06	59.38
Isoleucine	16.51	26.87	39.36	49.68
Tryptophan	0.39	1.98	0.61	4.24
Tyrosine	13.71	19.38	22.59	39.34
Methionine	6.33	9.01	16.70	19.71
Cysteine	1.92	1.76	2.50	5.43

1: Whey medium
2: Whey medium + 0.5% corn steep liquor
3: Whey medium + lactose + yeast extract
4: Whey medium + 0.5% corn steep liquor + 2% galactose

and 2% galactose was the best medium for growing *K. marxianus* to produce biomass containing the highest content of amino acids in comparison to the total amino acid content of the other media.

2.5. β-D-galactosidase production

The optimum conditions, obtained in the present work, for biomass production were applied, as preliminary experiments for the production of *β-D*-galactosidase. *K. marxianus* cells were grown on sweet and salted whey

Table 4

Biomass and β-galactosidase production at the optimum conditions with sweet and salted whey media

Treatment	Dry cells ($g l^{-1}$)		Total activity (μmol glucose per l)		Specific activity (μmol per g dry cells)	
	Sweet whey	Salted whey	Sweet whey	Salted whey	Sweet whey	Salted whey
Whey medium	9.11	5.15	14,780	10,200	1622.3	1980.58
Whey medium + yeast extract	15.15	7.71	16,200	11,520	1069.3	1494.10
Whey medium + yeast + + lactose	15.23	6.35	16,250	10,224	1066.9	1610.11
Whey medium + corn steep liquor	18.51	7.40	17,500	12,000	1149.0	1621.60
Whey medium + corn steep liquor + galactose	19.21	7.90	18,750	12,800	976.1	1620.30

One unit lactase activity (μ) is the amount of enzyme releasing 1.0 μmol of glucose per min at 35 °C.

media, with supplementation with $(NH_4)_2SO_4$ or corn steep liquor or yeast extract (0.5%). In addition whey medium was supplemented with yeast extract and lactose or corn steep liquor and galactose. The incubation was carried out at 35 °C and pH 4.5 for 5 days. Then the activity of the produced enzyme was determined. The results are given in Table 4. From these results, it can be observed that the supplementation of sweet and salted whey media with corn steep liquor or yeast extract or galactose and corn steep liquor increased the enzyme production. The addition of lactose (2%) to the whey medium supplemented with yeast extract did not improve the enzyme production.

It was found that, the addition of 10% salt to whey medium decreased the enzyme production about 25–30% by *K. marxianus* compared with sweet whey medium (Table 4), and the addition of corn steep liquor or yeast extract and galactose caused maximum production of the enzyme. These present results are similar to those recorded in *Candida pseudotropicalis* (MAGALY & CASTILLO, 1982)., *A. nidulans* (FANTES & ROBERTS, 1973), *K. fragilis* (DAVIES, 1956). In contrast DAN and SZABÓ (1973) and VITALIS and SZABO (1976) found that lactase production is induced by lactose but not by galactose. The stimulation effect of yeast extract and corn steep liquor on biomass and lactase production has been reported by DEBALES and CASTILLO (1979) as well as ROMANA-RAO and DUTTA (1981).

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INVESTIGATION INTO THE COMBINATION EFFECT IN TWO-COMPONENT WHEAT FLOUR MIXTURES FOR BAKING

K. HORVÁTH-ALMÁSSY[a] and F. ÖRSI[b]

[a]University of Horticulture and Food Industry, Faculty of Food Industry, H-6701 Szeged, Marx tér 7. Hungary

[b]Department of Biochemistry and Food Technology, Technical University, Budapest, H-1111 Budapest, Műegyetem rkp. 5—7. Hungary

The combination effect in baking was studied with two-component mixtures of wheat flours of different baking properties during three years.

Loaves were prepared of the flour mixtures according to the standard method, and the kneading properties were studied by Valorigraph measurements.

Evaluation of the data was carried out by principal component analysis.

Assuming the properties of the pure components utilized as additive, the values of the properties studied to be expected were calculated, and these were subtracted from the data measured. On the basis of principal component analysis of the differences obtained, it was established that the differences in loaves properties did not show any dependence on the year of harvest. On the contrary, the dough properties were dependent on the year.

The combination effect was expressed most markedly by the baking value according to TIBOR (1933) and, for the dough properties, by the dough formation time.

The baking value improved to a greater extent than expected in the cases when mixing prolonged the dough formation time. On the other band, dough formation time as shortened upon the influence of mixing, deteriorated the product quality as compared to the calculated values.

Keywords: wheat flour, blending of flour, baking quality, dough properties, additivity of flour characteristics

In bread manufacture the preparation of mixtures serves a double purpose (CORNELL, 1981).

On the one hand, the aim is to prepare by mixing the different components including a wheat flour according to a formula leading to a product of well-defined properties, namely the product bread, a commodity of loose and elastic structure with crisp brown crust from wheat flour, water and salt by adding yet other additives.

On the other hand, the wheat flour component of the formula can also be mixed from a range of wheat flours of different functional properties, in order to obtain, from the raw material available, a starting material of optimum quality.

In the literature the study of the combination effect in wheat flours as well as the analysis of practical introduction of the results to improvement and processing are mainly linked to the name of BOLLING (1977; 1980; 1982; 1983; 1986).

The quality improvement effects of mixing of different German wheat cultivars were investigated by BOLLING and MEYER (1982). Their experiments proved, that the mixing of flours is a proper method to optimize the rheological properties of dough, and to achieve higher loaf volumes.

In order to study the preparation of a basic material of optimum quality, we investigated the combination effect, of two-component mixtures of flours, of defined wheat cultivars with regard to breadmaking properties in a series of experiments carried out for several years. Some part of our results were published earlier (HORVÁTH-ALMÁSSY, 1986; 1989, HORVÁTH-ALMÁSSY et al. 1990; HORVÁTH-ALMÁSSY & ÖRSI, 1990).

1. Materials and methods

1.1. Materials

Two component flour mixtures were prepared in the mass ratios of 75 : 25, 50 : 50 and 25 : 75 from wheat flours of identical varieties harvested in 1986, 1987 and 1988, respectively. One of the components of the mixture was always the variety Yubileynaya 50 or the variety GK Öthalom, both belonging to quality group A.

The homogeneity of the mixtures was ensured by triple sieving.

The composition of the varieties tested in the individual years is given in Tables 1 and 1a.

The samples were obtained from the Kiszombor plantation of the Szeged Research Institute for Growing of Grain Crops.

1.2. Methods

Test loaves were baked of the basic flours and the mixtures according to the HUNGARIAN STANDARD (1971) using the amount of water which corresponded to the water uptake capacity as determined in the Valorigraph.

Volume and form quotient of the test loaves were measured, and the baking number according to TIBOR (1933), a contracted measure of loaf quality was calculated.

According to TIBOR (1933), in evaluating the test loaf volume and form quotient must be taken into account jointly as follows: the values for bread volume and bread form are taken from the TIBOR (1933) made Table, and the arithmetical mean of these values gives the baking value.

The Table has been compiled on the basis of practical experience of many years. If defines in a percentage scale as 100%, when the bread volume amounts to 520 cm^3 per 100 g flour and as 50% if it is 270 cm^3 per 100 g. For the form quotient the value of 1.60 is defined as 100% while that of 3.20 as 50%.

Table 1

Distribution of basic wheat varieties in the individual years

Basic varieties	Year	Symbol[a]	Quality group
Yubileynaya	1986		A2
	1987		A2
	1988		A2
GK Öthalom	1987		A1
	1988		A2
Martonvásári 8	1986		B1
	1987		B1
	1988		B2
Martonvásári 14	1988		C2
GK Ságvári	1987		C1
	1988		B2
Baran'ka	1986		B1
	1987		B1
GK Csilla	1986		B2

[a] In the Figures the symbol of the variety of inferior quality was consequently used for the mixtures.
1986: n = 9, 1987: n = 21, 1988: n = 11.
Total number of samples including basic flours n = 55.

Kneading properties were studied by Valorigraph tests according to HUNGARIAN STANDARD (1973). Baking value, time of dough formation, dough stability, the sum of dough formation and stability time as well as the time of total dough stability were determined. The latter parameter is the time lapse, during which the upper edge of the valorigram remains above the consistency line of 500. According to experience, this is a more characteristic value than the baking value as it does not take into account the apparent quality deterioration originating from the possible fluctuations of consistency occurring during the test (SIETZ, 1985).

The test loaves were prepared at the Quality Control Laboratory of the Baking Company of Country Csongrád. Valorigraph tests were performed at the Quality Control Laboratory of the Tisza Mill, Szeged.

Table

Primary quality properties

	1986				
	loaf volume (cm^3)	valorigraph value	wet gluten (%)	gluten sreading (mm)	falling number (sec)
			YUBILEYNAYA 50		
Sample size	2	2	2	2	2
Average	1200	83.65	31.65	3.75	392.5
Standard error	20	0.95	0.15	0.25	5.5
Minimum	1180	82.7	31.5	3.5	387
Maximum	1220	84.6	31.8	4	398
			MARTONVÁSÁRI 8		
Sample size	2	2	2	2	2
Average	930	62.5	31.25	5.35	411
Standard error	20	2.1	0.25	0.15	17
Minimum	910	60.4	31	5.2	394
Maximum	950	64.6	31.5	5.5	428
			BARAN'KA		
Sample size	2	2	2	2	2
Average	930	64.3	31.4	3.75	408.5
Standard error	30	0.9	0.1	0.25	6.5
Minimum	900	63.4	31.3	3.5	402
Maximum	960	65.2	31.5	4	415
			GK CSILLA		
Sample size	2	2	2	2	2
Average	850	45.35	35.75	11.5	769
Standard error	10	0.65	0.25	1.5	4
Minimum	840	44.7	35.5	10	765
Maximum	860	46	36	13	773
Sample size					
Average					
Standard error					
Minimum					
Maximum					

1.3. Method of evaluating the results

For characterizing the properties of both the basic flours and the mixtures, principal component analysis was used (SVÁB, 1979).

Assuming that after mixing the properties of the pure components used were additive, the values to be expected for the properties tested of the mixtures were calculated and subtracted from the values measured. From the differences thus obtained — including separately the kneading properties and the loaf properties — contracted variables were formed by principal component analysis.

1a

of identical varieties

1987					1988				
loaf volume (cm^3)	valori-graph value	wet gluten (%)	gluten spreading (mm)	falling number (sec)	loaf volume (cm^3)	valori-graph value	wet gluten (%)	gluten spreading (mm)	falling number (sec)
	YUBILEYNAYA 50					YUBILEYNAYA 50			
2	2	1	2	—	4	4	6	2	6
1225	83.9	33.5	3.95	—	1100	74.98	36.45	5.75	371.6
25	1.4	0	0.05	—	16.8	1.85	0.93	0.25	6.38
1200	82.5	33.5	3.9	—	1060	69.9	33.3	5.5	341
1250	85.3	33.5	4	—	1140	78.8	38.3	6	383
	GK ÖTHALOM					GK ÖTHALOM			
2	2	2	2	—	2	2	2	2	2
1205	81.05	33	4.05	—	1100	68.5	30.95	3.35	397
15	0.25	0.5	0.05	—	10	1	0.35	0.15	7
1190	80.8	32.5	4	—	1090	67.5	30.6	3.2	390
1220	81.3	33.5	4.1	—	1110	69.5	31.3	3.5	404
	MARTONVÁSÁRI 8					MARTONVÁSÁRI 8			
2	2	1	2	—	2	2	2	2	2
810	61.15	34	3.75	—	940	58.3	34.9	3.75	320.5
10	0.35	0	0.15	—	10	1.5	0.9	0.25	2.5
800	60.8	34	3.6	—	930	56.8	34	3.5	318
820	61.5	34	3.9	—	950	59.8	35.8	4	323
	BARAN'KA					MARTONVÁSÁRI 14			
2	2	2	2	—	2	2	3	2	3
875	61.8	31.5	4.3	—	905	39.5	37.06	8.5	347.6
25	1.6	0.5	0.2	—	15	1.5	0.23	0.5	4.41
850	60.2	31	4.1	—	890	38	36.7	8	341
900	63.4	32	4.5	—	920	41	37.5	9	356
	GK SÁGVÁRI					GK SÁGVÁRI			
2	2	2	2	—	2	2	3	2	3
800	31.95	34	7.25	—	895	47.3	28	5.25	367.6
10	1.15	0.4	0.25	—	15	0.3	0.3	0.25	7.31
790	30.8	33.6	7	—	880	47	27.4	5	358
810	33.1	34.4	7.5	—	910	47.6	28.3	5.5	382

The observed variables included in the evaluation were as follows:
Difference in kneading properties:

Valorigraph value number	(Val)
Dough formation	(DF)
Dough stability	(DS)
T 2 + 3	(Sum)
Total dough stability	(TDS)

Difference in loaf properties:

Loaf volume	(LV)

Form quotient	(FQ)
Baking number by Tibor	(BN)

The first and second principal component values of the differences in properties of the observation units, i.e. the flour mixtures were presented in a coordinate system.

Presentation was performed according to years and groups of properties (dough, loaf).

2. Results

2.1. Interpretation

2.1.1. Characterization of basic flours. The qualitative heterogeneity of the basic flours with respect to kneading and loaf properties are shown in Figs. 1a and 1b as illustrated by the contracted variables obtained upon principal component analysis.

The observation variables were included in the evaluation as presented in para 1.3.

The interpretation of the original variables is the same as described in para 1.3.

Symbols:

MCIPO3.PCO1: first principal component
MCIPO3.PCO2: second principal component
MCIPO3.ev: year of harvest of the samples
Figs. 1a; 1b; 1c; 1d

From Figs. 1a–d it is obvious that, according to the first two principal component variables of both the dough properties and the loaf properties, the flour samples originating from the same variety can be essentially subdivided into three groups. The first group is composed of Yubileynaya 50 and GK Öthalom, irrespective of the year of harvest. These samples are located in the quarter +; + of the first two main component variables. It was expected of these varieties to possess a so-called mixing value, i.e. that they would alter the quality of the rest of the varieties to a significantly greater extent than additivity.

The other, well separated group is formed by the flours of low baking quality (1986: GK Csilla; 1987: GK Ságvári; 1988: Martonvásári 14). These fell, in both of the Figures, into the quarter +; —, far from the axis of the second principal component variable.

The third group is formed by the rest of pure components, characterized by medium flour quality. They are to be found mainly along the axis of the second principal component, in the first place in the quarters —; and —; —.

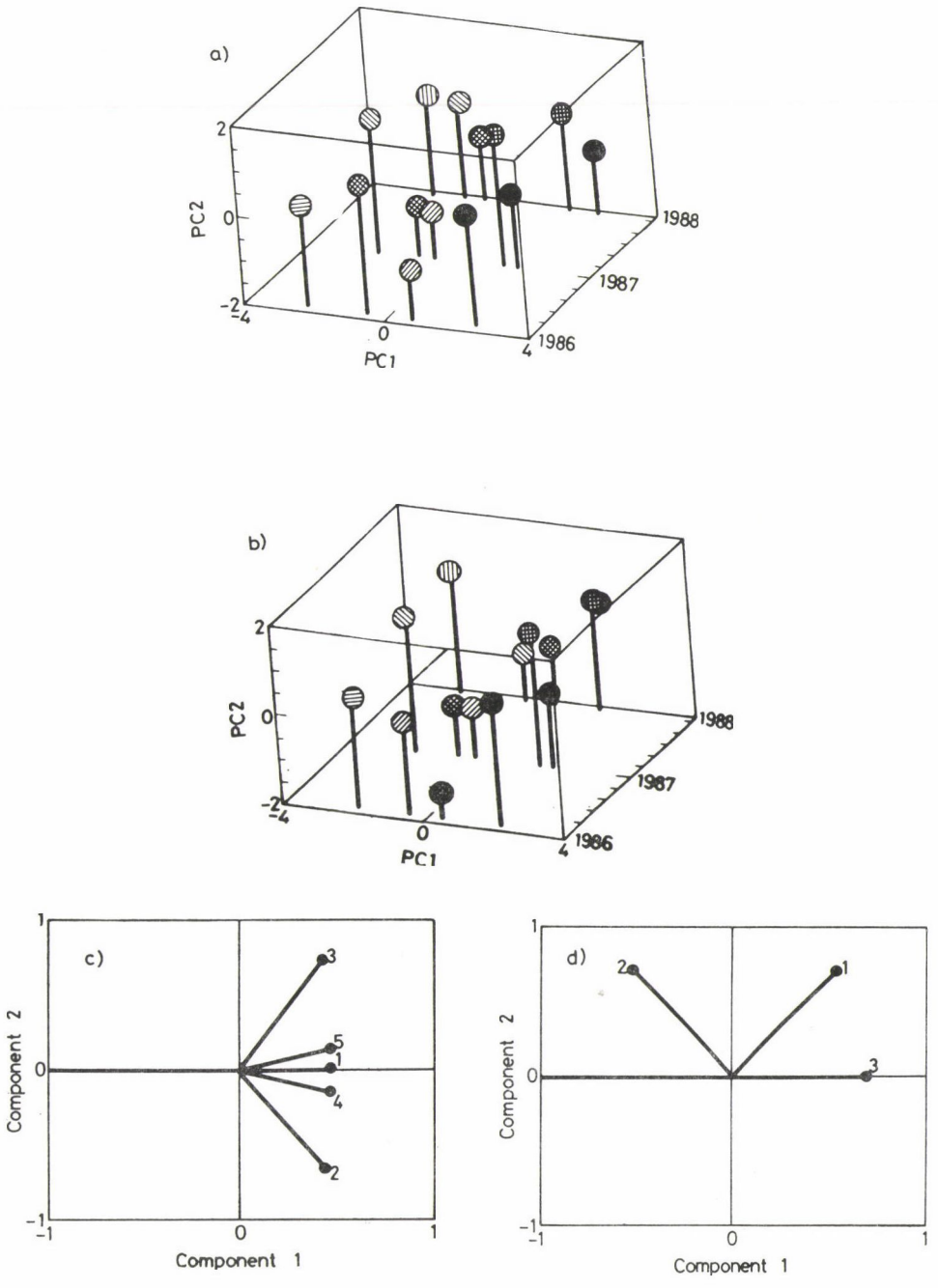

Fig. 1. Principal component analysis of the original variables according to years a) Dough properties; b) Loaf properties; c) The projection of the vectors of dough properties; d) The projection of the vectors of loaf properties
PC1: first principal component variable; PC2: second principal component variable

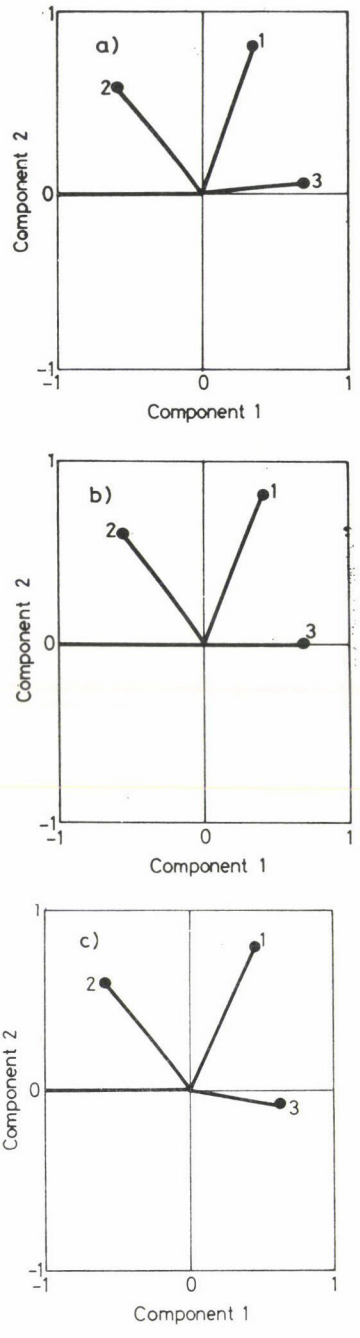

Fig. 2. Principal component analysis of the differences in loaf properties. Projection of the original variables in the plane of the principal components
a) 1986; b) 1987; c) 1988
Difference variables: 1: (Vol); 2: (FQ); 3: (BN)

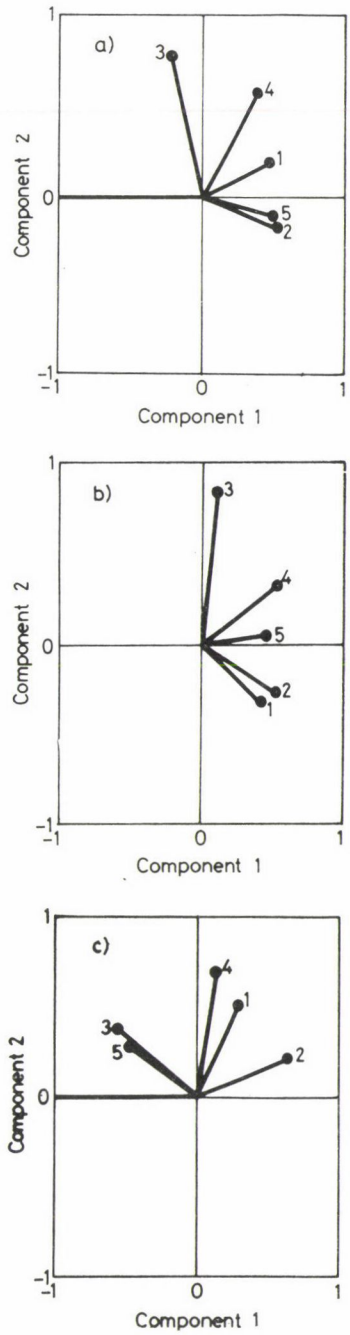

Fig. 3. Principal component analysis of the differences in kneading properties. Projection of the original variables in the plane of the principal components
a) 1986; b) 1987; c) 1988
Difference variables: 1: (Val); 2: (DF); 3: (DS); 4: (Sum); 5: (TDS)

2.2. Principal component analysis of the differences between the observation variables

The first two principal components are responsible, on the average for 79.28% of the total variance of the kneading properties and 98.76% of the total variance of loaf properties.

The projections of the original difference variables in the plane of the principal components are shown in Figs. 2a–c and 3a–c.

Table 2

The weight of kneading difference variables in the first two principal components

Year	Decisive observation variables			
	PC1	(%)	PC2	(%)
1986	Val	24.2	DS	60.2
	DF	29.4	TDS	32.0
	Sum	26.4		
1987	Val	18.3	DS	71.9
	DF	28.4		
	Sum	29.5		
	TDS	22.8		
1988	DF	40.0	Val	24.8
	DS	30.5	Sum	47.6
	TDS	21.2		

The differences in loaf properties give identical results in all the three years of harvest (Figs. 2a–c), i.e. the first principal component is primarily determined by the baking value according to TIBOR (1933) while in the second principal component the weight of the difference in loaf volume and form quotient are the greatest.

In contrast, the deviation of kneading properties from additivity shows a considerable dependence on the year of harvest (Figs 3a–c). A summary of this is given in Table 2.

The results permit of the conclusion that the correlation between loaf properties is independent of the year of harvest while the differences in kneading properties are due to reasons varying with the year of harvest. With the latter, the samples of 1986 and 1987 show essentially a similarity, save for the slight difference in the values of TDS. In contrast, the grouping of the differences in the 1988 samples is fundamentally different.

In the first two years the differences in Valorigraph values were related to the differences in dough formation time. The correlation of the sum (2 + 3) with these values suggests that the difference in dough stability is not considerable.

In the case of the year 1988 an essential modification could be observed. The variation of the Valorigraph value (Val) is not in the same group, i.e.,

it does not correlate with the variation of dough formation. The variation of Valorigraph values forms a common group with the sum (Sum) values. This is made possible by the fact that the variation of the values DF and DS upon the action of mixing occurs in the opposite direction (cp. Fig. 3c).

However, in all the three years, the differences in dough formation time DF play an important part in creating the combination effect.

2.3. Presentation of the observation variables in the planes PC1; PC2

When presenting the values of the first two principal component variables obtained as described in para. 3, separately for the individual years, Figs. 4a–e and 5a–e were obtained.

The confidence intervals related to PC1 (first principal component variable) and PC2 (second principal component variable), respectively, were marked as well.

From the Figures it can be seen unambiguously that values differing significantly from those calculated were obtained, i.e., the combination effect took place.

The trend in the variation shows a strong dependence on the year, both with the kneading and with the loaf properties.

2.3.1. Interpretation of the variation of loaf properties. In Figs. 4a, 4d and 4e it can be seen that, in general, similar changes occurred with the flours of the years 1986 and 1988. Exceptions are the mixtures Yubileynaya 50–Martonvásári 8 as well as — out of the mixtures Yubileynaya 50–Martonvásári 14 — the flour mixture of the composition 25 : 75.

Contrary to this, although the observation units are located also in this case along the PC1, in 1987 points can be found in both the positive and the negative direction. It is interesting to note that the mixtures of Yubileynaya 50 and GK Öthalom behaved in a different way, this applies particularly to their mixtures with GK Ságvári. This can be explained by the differences of these two varieties of A quality in 1987 (cf. Figs. 1a, b).

2.3.2. Interpretation of the differences in kneading properties. Taking into account the weights of the principal component variables (Table 2), the changes in kneading properties can be interpreted as shown in Figs. 5a–e.

The difference in the kneading properties of the two years (1986 and 1987) similarly with respect to loaf properties, follows an opposite trend as seen in Figs. 5a, 5b and 5c. While with the samples of 1986 the observation units are located in the direction of the positive half-axis of the first principal component (with the exception of the mixture 75 : 25 of Yubileynaya 50–Baran'ka), the 1987 samples can be mainly found along the PC1. This means that in the differences of kneading properties, the differences in dough stability do not play any part — apart from some exceptions.

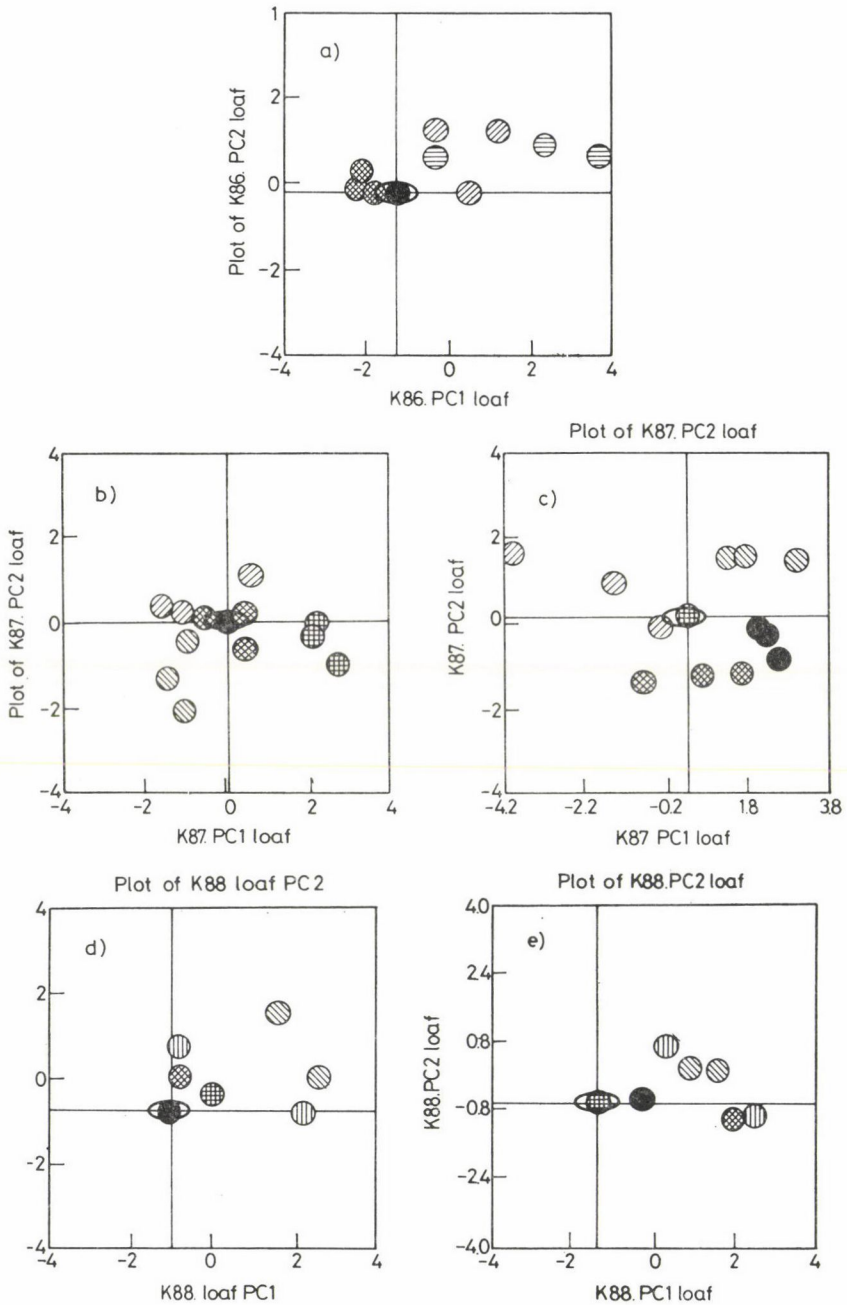

Fig. 4. Presentation of the observation variables in the plane of the first two principal components. Loaf properties.
a) 1986. Mixtures of Yubileynaya 50; b) 1987. Mixtures of Yubileynaya 50; c) 1987. Mixtures of GK Öthalom; d) 1988. Mixtures of Yubileynaya 50; e) 1988. Mixtures of GK Öthalom

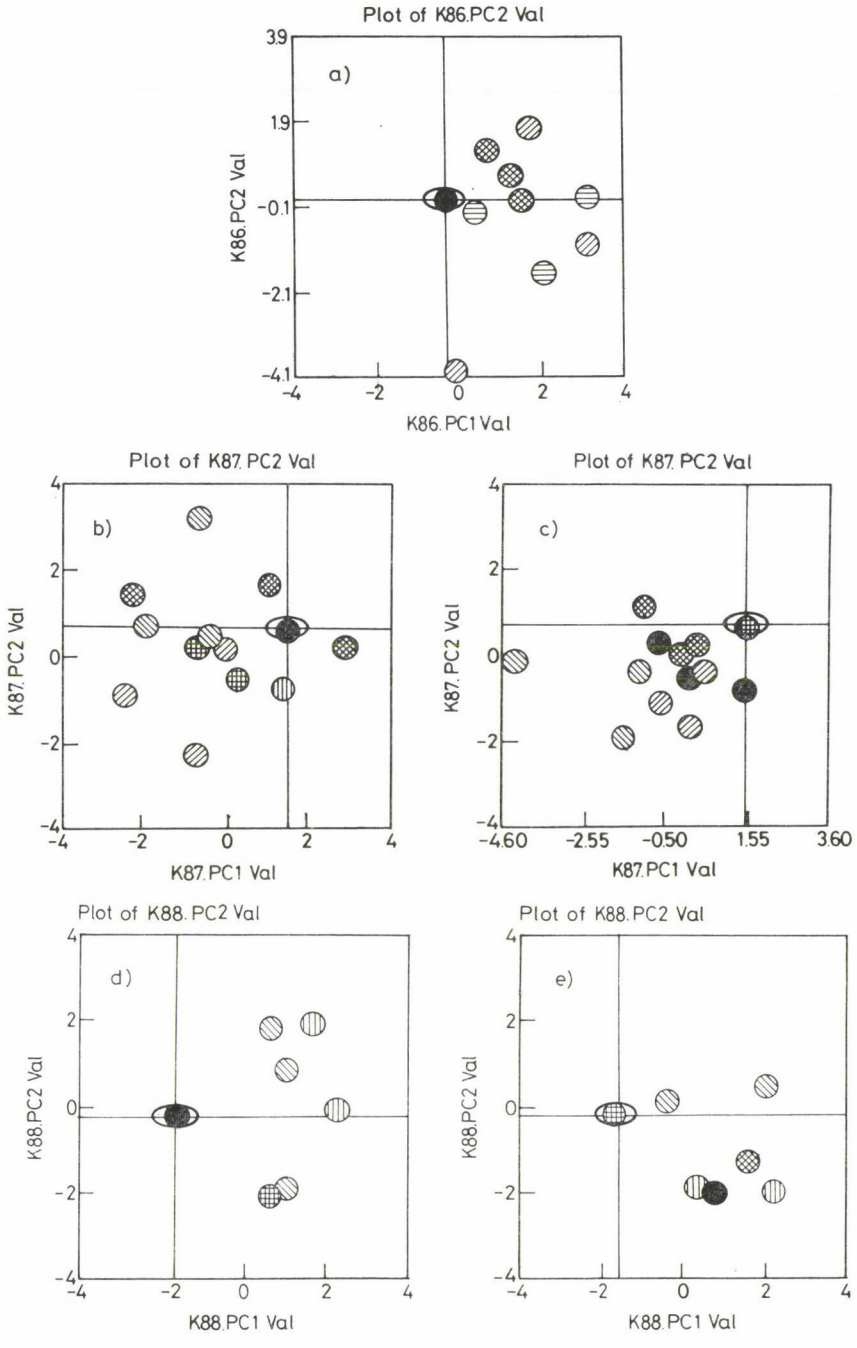

Fig. 5. Presentation of the observation variables in the plane of the first two principal components. Kneading properties.
a) 1986. Mixtures of Yubileynaya 50; b) 1987. Mixtures of Yubileynaya 50; c) 1987. Mixtures of GK Öthalom; d) 1988. Mixtures of Yubileynaya 50; e) 1988. Mixtures of GK Öthalom

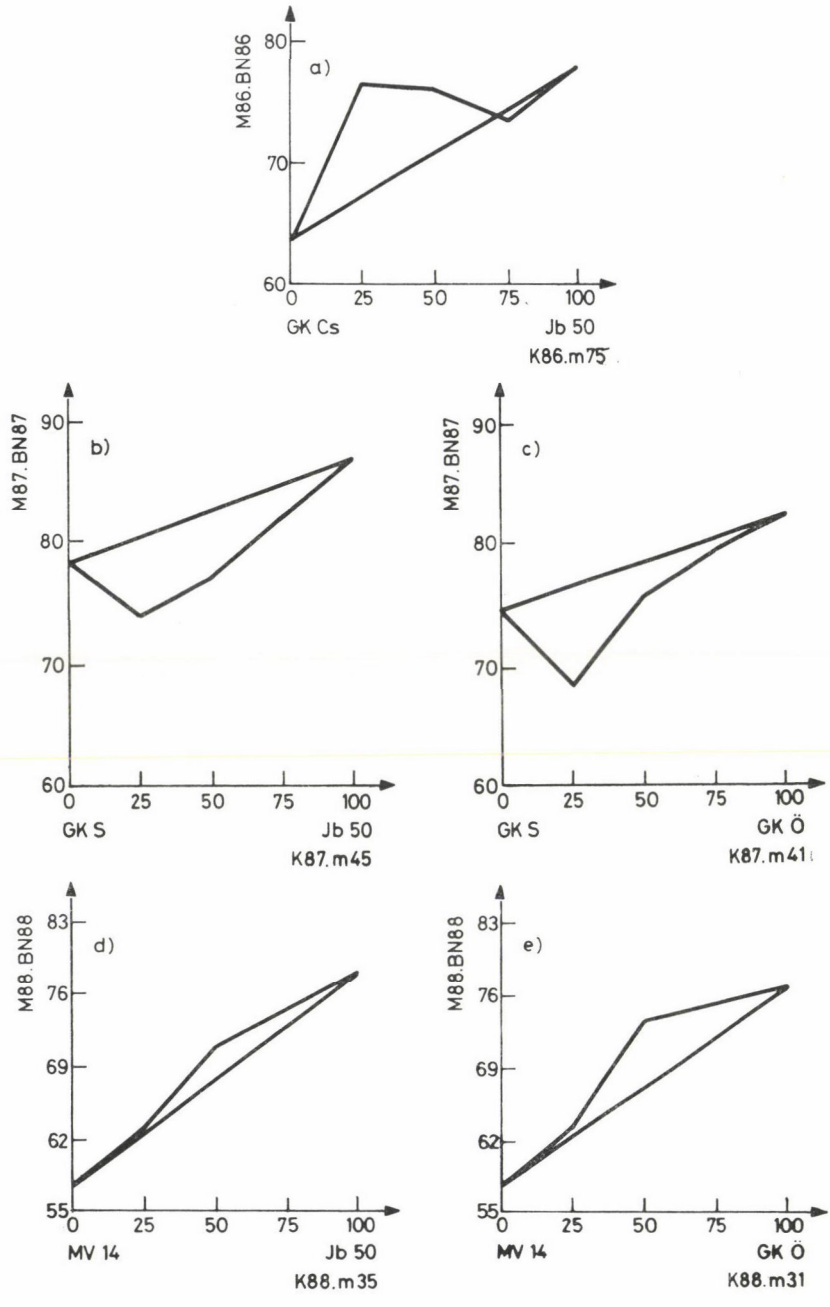

Fig. 6. Dependence of the baking value according to Tibor on the composition of individual mixtures
a) 1986. GK Csilla — Yubileynaya 50; b) 1987. GK Ságvári — Yubileynaya 50; c) 1987. GK Ságvári — GK Öthalom; d) 1988. Martonvásári 14 — Yubileynaya 50; e) 1988. Martonvásári 14 — GK Öthalom

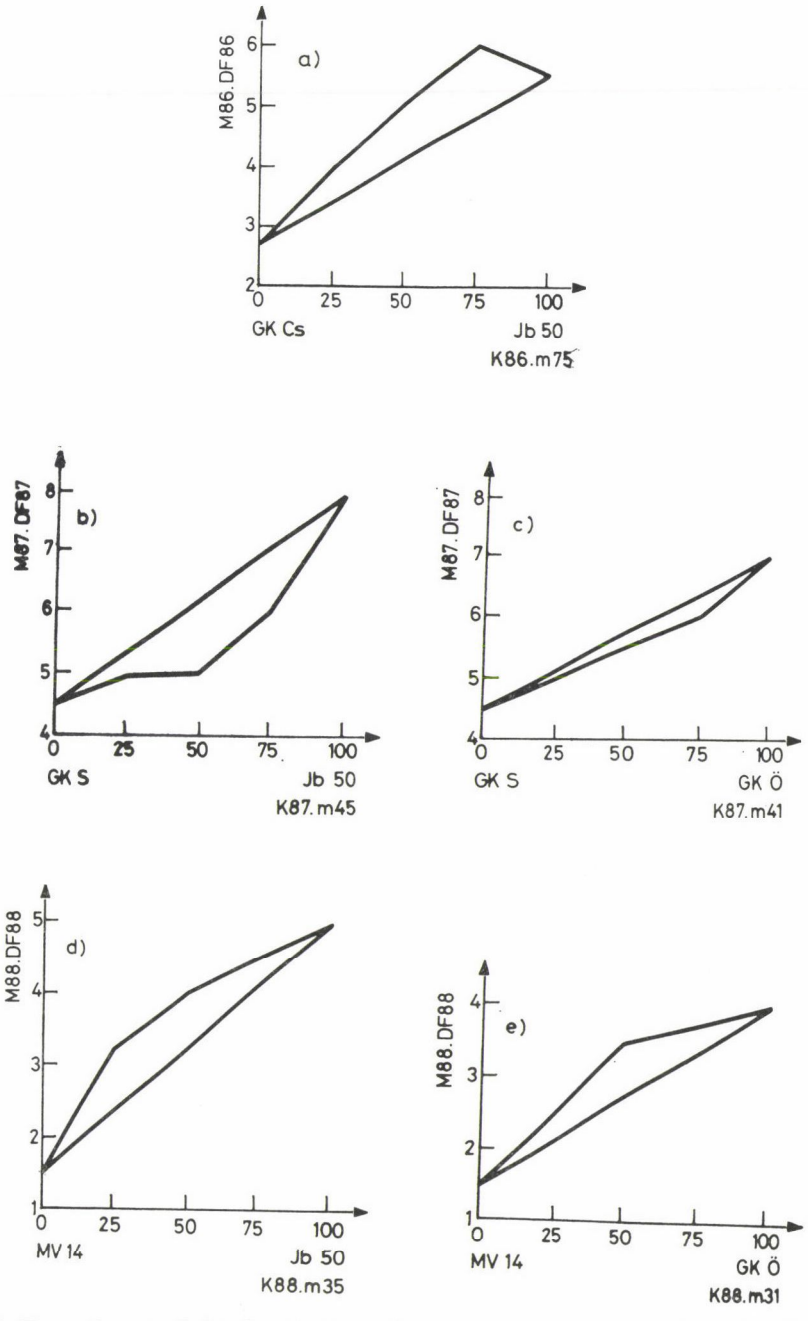

Fig. 7. Dependence of the dough formation time on the composition of individual mixtures
a) 1986. GK Csilla — Yubileynaya 50; b) 1987. GK Ságvári — Yubileynaya 50; c) 1987. GK Ságvári — GK Öthalom; d) 1988. Martonvásári 14 — Yubileynaya 50; e) 1988. Martonvásári 14 — GK Öthalom

From the presentation of the principal component variables of the 1988 observation units it can be seen that the difference between the samples are mainly due to the differences in DF and DS. The points representing the mixtures are located along the positive half-axis of PC1, i.e., the differences are of identical character in the mixtures.

2.4. The original variables and the combination effect

On the basis of the principal component analysis it was found that the combination effect is shown in the most pronounced way by the baking value according to TIBOR (1933) and the time of dough formation. In order to illustrate this, the behaviour of the mixtures of the wheat varieties Yubileynaya 50 and GK Öthalom with partners of poor quality was plotted as example as a function of composition. Figs. 6a–e and 7a–e demonstrate the combination effect as expressed by the Tibor value and the time of dough formation, respectively.

From the Figures it can be seen that an improvement in baking value occurred in the cases when mixing prolonged the time of dough formation. On the other hand, the decrease in dough formation time as it occurred upon the influence of mixing, deteriorated the quality of the product as compared to the calculated value. This corresponds in baking technology to the kneading tolerance of wheat.

3. Conclusion

According to our experimental results, we concluded that in optimization of baking quality and rheological properties of flours the improving effects of combination pay a very important role.

The knowledge of these results can be useful in the standardizing of quality of industrial wheat flours without artificial additives.

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STERILIZATION OF SPICES AND VEGETABLE SEASONING BY GAMMA RADIATION

G. LESCANO, P. NARVAIZ and E. KAIRIYAMA

Comision Nacional de Energia Atomica Gerencia de Area Radioisotopes y Radiaciones (1842) Agencia Minipost-Centro Atomico Ezeiza, Buenos Aires. Argentina

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Ginger, turmeric, Cayenne pepper, onion and garlic powders were gamma irradiated with doses of 10 and 30 kGy, and stored at room temperature for six months. Microbiological, chemical and sensory analyses were performed. Samples were sterilized with 30 kGy.

The pH values of water extracts of these condiments appear practically unchanged by the applied gamma radiation doses. Gas-chromatographic patterns of ginger (volatile oil and oleoresins) and Cayenne pepper (volatile oil) in control and irradiated samples were observed in irradiated samples though, in general, without significant differences.

Spectrophotometric analysis of methyl alcohol extracted of the pigments showed no irradiation effect in turmeric and ginger. Colour loss was observed in Cayenne pepper and onion powder, proportional to radiation dose soon after irradiation; differences between control and irradiated samples became lower at the end of the storage period.

Seasoning capacity (odour, flavour and pungency) were not altered by 30 kGy. Colour was perceived as different in ginger and Cayenne pepper (slightly lighter), and in onion and garlic powders (slightly darker), but not in turmeric, which is used to give colour.

Keywords: gamma radiation, sterilization, spices, vegetable seasonings

Spices and vegetable seasonings are generally heavily contaminated, predominantly with aerobic sporeforming bacteria and moulds, which are source of spoilage in the foods in which they are incorporated. Microorganisms of public health significance such as *Salmonella*, *Escherichia coli*, *Clostridium perfringens*, *Bacillus cereus* and toxigenic moulds can also be present (WHO, 1989).

Good manufacturing practices during harvest and processing could improve their hygienic quality, but frequently not to such extent as to reach an acceptable microbiological level (WHO, 1989).

These heavily contaminated dry ingredients cause serious troubles in the food processing industry. Research has already shown that gamma radiation is effective to reduce microbial load, without causing any significant organoleptic or chemical alteration (TJABERG et al., 1972; FARKAS et al., 1973; KISS et al., 1978; FARKAS 1973, 1983, 1985, 1987; ITO et al., 1985; MOSSEL 1985; also experiences of our own; KAIRIYAMA et al., 1988; NARVAIZ et al., 1989). When these products are incorporated into manufactured foodstuffs

submitted to low heat treatment or even without it like some sauces and dressings which are stored at room temperature, they should be sterilized.

The present work has been carried out on 3 spices and 2 vegetable seasonings, provided by a local industrial manufacturer of sauces and dressings, who wished to improve the hygienic quality of the ingredients, so as to extend the shelf life of their products. Most of studies on the chemical and sensory aspects of spices irradiation have been carried out with doses up to 10 kGy. The Argentine Alimentary Codex Commission has approved spices irradiation up to a dose of 30 kGy (KAUPERT, 1988), as well as other countries do, i.e.: USA and Taiwan JAEA, 1990. So, the purpose of this work was to verify the effect of this high radiation dose on sterilization, and the chemical and sensory quality of the analyzed ingredients.

1. Materials and methods

1.1. Materials

Studies were performed on: ginger (*Zingiber officinale* Roscoe) from Sri Lanka, turmeric (*Curcuma longa*, Linn) from China, all of them ground; and on onion (*Allium cepa* Linn) and garlic (*Allium sativum* Linn) both in powder, of Argentina.

Approximately 300 g each of the spices and vegetable seasonings were packed in polyethylene bags, of 100 μm thickness, containing 5 g each.

1.2. Irradiation

The samples were irradiated in a ^{60}Co Semi Industrial Facility in the Ezeiza Atomic Center. The activity of the source was 1.11×10^{16} Bq (February/88). The dose rate was 5.38 kGy h^{-1} determined with the routine dosimeter potassium nitrate, referred to Fricke dosimeter (DORDA et al., 1989). Gamma radiation doses of 10 and 30 kGy were applied at room temperature. The relationship between minimum and maximum absorbed doses was 1.15.

1.3. Storage

Control and irradiated samples were stored in the darkness at room temperature (20 °C $\pm$ 5 °C) during six months.

Microbiological, chemical and sensory evaluations were performed in the first and sixth month of storage.

1.4. Microbiological tests

Samples of 2–3 g were taken from 3 bags on each analysis date. Serial decimal dilutions were made with peptone water (0.1% w/v) and duplicate inoculums of 0.1 cm^3 were spread on every culture medium plate used.

The following microbial counts were determined:

Total aerobic bacteria count: at 33 °C, for 4 days in triptone-glucose, yeast extract-agar medium (10.0, 5.0, 3.0, 15.0 g dm^{-3}; respectively, plus sodium chloride 5.0 g dm^{-3}, pH 7).

Sulfite reducing clostridia: in Wilson Blair medium at 33 °C, for 3 days.

Sporeformer aerobic bacteria: as in a previous work (NARVAIZ et al., 1989).

Enterobacteriaceae: as in previous work (NARVAIZ et al., 1989).

1.5. Chemical tests

Sampling: material was taken from 3–5 bags on each analysis date. Triplicate determinations were carried out on control and both irradiated samples.

Results were statistically evaluated by means of a Student t test for mean values, $P \leq 0.05$.

The following determinations were performed:

— *pH:* 0.25 g samples were blended with 25 cm^2 of water for 15 min, with occasional stirring, then they were filtered through paper (SS 589[1]), and measured in a pH-meter. This determination was carried out 10 days after irradiation.

— *Colour:* 0.1 g samples were blended with 5 cm^3 of methyl alcohol for 15 min, with occasional stirring, filtered through paper (SS 589[1]), and immediately read in a spectrophotometer.

Curcuma colour was also analysed by thin-layer chromatography. Ethyl alcohol extracts (PRUTHI, 1980) were run on plates coated with silicagel G, 25 μm thickness, with butyl alcohol saturated with ammonia as running solvent. Colour intensities of control and irradiated spots were compared visually, under daylight and ultraviolet light (366 nm).

These determinations were carried out 20 and 180 days after irradiation.

— *Essential oils:* Volatile oils were separated by steam distillation. In ginger this was carried out on the residue resulting from diethyl-ether extraction of the spice in Soxhlet apparatus, and both volatile oil and resin were obtained. Samples were analysed by gas chromatography (PRUTHI, 1980). Column: 10% Carbowax 20 M; FID; Column temperature: 90–230 °C, 4 °C min^{-1}.

The determinations were performed 60 days after irradiation.

1.6. Sensory test

Sensory rating was carried out by a selected and trained panel composed of 13 judges (8 women and 5 men) (MACKEY et al., 1954).

Triangle test with characterization of difference was conducted in duplicate. The significance of the results was determined by a table of numbers of correct identification for significance in triangle test, $P \leq 0.05$ (ASTM, 1968).

The assays were performed in the following way:

— Directly on the samples: colour in all of them, and odour of ginger.

— On samples dispersed in lean white cheese in different concentrations, the seasoning capacity was evaluated: Pungency and flavour of ginger, concentration of 5%; pungency of Cayenne pepper, concentration of 1%, odour and flavour of onion and garlic, concentration of 1% and 2%, respectively.

The triangle tests were performed on control and 30 kGy samples. If these results were significantly different, control and 10 kGy samples were also evaluated.

2. Results and discussion

2.1 Microbial test results

Table 1 presents data of microbiological tests on control and 10 kGy samples.

It can be seen that 10 kGy reduced total aerobic bacterial count by 2–4 log cycles, reaching levels below 4 log CFU g^{-1} (colony forming units per g). Our regulation authorizes 30 kGy to attain sterility; with this dose no surviving aerobic bacteria were detected in 1 g. Sporeforming bacteria incidence in the total number of aerobic bacteria was high: more than 92% in ginger, Cayenne pepper and turmeric, and more than 97% in onion and garlic powders.

Sporeforming aerobic bacterial count was reduced by 2–5 log cycles with 10 kGy, and no survivors were found in 1 g with 30 kGy.

Sulfite reducing clostridium counts of every control sample were between 1.69 and less than 1 log CFU g^{-1}, and that of *Enterobacteriaceae* less than 1.69 log CFU g^{-1}.

2.2. Chemical tests

The results of the pH determination are shown in Table 2. Significant differences from the control sample were only observed in turmeric and garlic. This test was carried out as a requirement of the manufacturer, who intended to add the condiments to a liquid medium in which great pH variations would be undesirable.

Table 1

Microbial contamination of control and 10 kGy spices and vegetable seasoning on 1st and 6th storage months

Samples	Analysis date (storage month)	Logarithm of Colony Forming Unit g^{-1}					
		TABC		SABC		MYC	
		0.0 kGy	10.0 kGy	0.0 kGy	10 kGy	0.0 kGy	10 kGy
Ginger	1st	6.56	2.69	6.07	1	2.17	1.69
	6th	6.67	2.69	6.30	1	2.39	1.69
Cayenne	1st	6.88	2.69	6.43	3.30	2.69	1.69
Pepper	6th	7.00	3.20	6.46	1.69	3.39	1.69
Turmeric	1st	6.14	3.60	5.78	3.60	3.17	1.69
	6th	6.17	2.69	6.49	2.0	2.74	1.69
Onion	1st	3.84	1.69	3.00	1.69	2.0	1.69
Powder	6th	3.54	1.69	3.63	1.69	2.47	1.69
Garlic	1st	6.17	2.69	5.04	2.69	3.65	1.69
Powder	6th	6.32	2.00	5.51	1.67	3.34	1.69

TABC: total aerobic bacteria count
SABC: sporeformer aerobic bacteria count
MYC: mould and yeast count

Table 2

pH values of control and irradiated water extracts, on the 10th storage day

Sample	Radiation dose (kGy)		
	0	10	30
Cayenne pepper	5.45 ± 0.02	5.46 ± 0.03	5.48 ± 0.02
Ginger	6.70 ± 0.06	6.81 ± 0.03	6.66 ± 0.03
Turmeric	6.39 ± 0.06	6.2 ± 0.1*	6.21 ± 0.02*
Garlic powder	6.38 ± 0.01	6.23 ± 0.003*	6.251 ± 0.002*
Onion powder	6.1 ± 0.1	6.03 ± 0.04	5.98 ± 0.05

*: significantly different from control at $P \leq 0.05$ probability level; $n = 3$

Table 3

Absorbance values of methyl alcohol extracts of control and irradiated turmeric and ginger samples (wavelenghts: 425 and 350 nm, respectively), along storage time

Sample	Storage time (days)	Radiation dose (kGy)		
		0	10	30
Turmeric	20	0.20 ± 0.02	0.20 ± 0.02	0.22 ± 0.02
	180	1.8 ± 0.1	2.4 ± 0.6	2.0 ± 0.2
Ginger	20	1.50 ± 0.05	1.30 ± 0.05*	1.45 ± 0.05
	180	0.44 ± 0.01	0.46 ± 0.01	0.44 ± 0.04

*: significantly different from control at $P \leq 0.05$ probability level; $n = 3$

Regarding colour analysis, Table 3 shows the results obtained with turmeric and ginger. For turmeric there were no significant differences among control and irradiated samples in the absorbance intensity. On the 20th day of storage a slight shift of the peak of irradiated samples was observed (425 nm (control sample) → 415 nm (irradiated samples)). On the 180th day of storage practically no peak was found, only an outline in the control sample; nevertheless absorbance values at 425 nm were recorded. They were higher than those of the 20th storage day, probably due to enhanced extractability; the bags containing turmeric were greasy at the end of the storage period.

Thin-layer chromatography showed, under visible light, 4 spots, and under ultraviolet light (366 nm), 6 spots. No differences among samples were found through visual comparison of R_f, shape and colour of spots.

For ginger, maximum absorbance was found at 350 nm. Initially, diminished values were observed in the irradiated samples; with storage time, no significant differences could be found, and the absorbance intensity was remarkably lower in every case.

Methanolic extracts of Cayenne pepper showed an absorbance peak at a wavelength of 425 nm. A significant diminution of the intensity of the extracted pigments was observed due to irradiation, this effect being more evident at the beginning of storage time. At the end of the storage period, as in ginger, the absorbance intensity was remarkably lower in every case, as has also been observed by FARKAS and co-workers (1973) in ground paprika, and differences between control and irradiated samples were less pronounced. Results are summarized in Fig. 1.

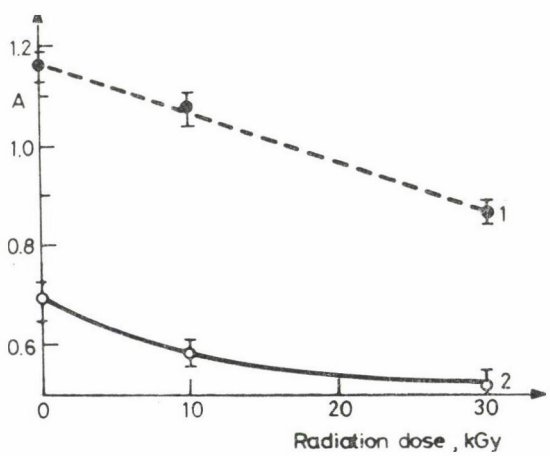

Fig. 1. Absorbance values of methyl alcohol extracts of Cayenne pepper, as a function of radiation dose.

1: 20th day of storage; 2: 180th day of storage. Control sample is always significantly different from the irradiated ones. Vertical segments indicate standard deviation

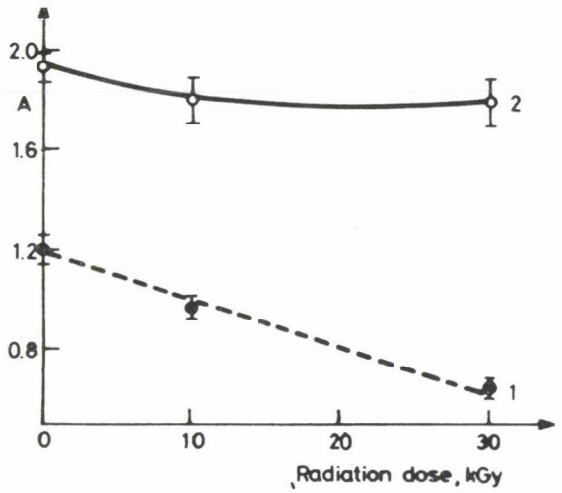

Fig. 2. Absorbance values of methyl alcohol extracts of onion powder, as a function of radiation dose. Control sample is significantly different from the irradiated ones only on the 20th day of storage
1: 20th day of storage, 2: 180th day of storage. Vertical segments indicate standard deviations

Figure 2 shows the results obtained with onion powder. Initially, an absorbance peak was found at 370 nm in the control samples, which shifted slightly to 365 and 360 nm, for the 10 and 30 kGy samples, respectively. Diminution of the intensity of the extracted pigments was found due to irradiation, with significant differences at the beginning of storage, this effect being more pronounced with increasing radiation doses. Values at the end of the storage period were higher, probably due to browning, without significant differences among samples.

The garlic powder samples did not show an absorbance peak in the visible region.

These results in general suggest that when there was destruction of characteristic pigments due to gamma radiation, it followed a rather linear pattern with radiation dose soon after the process took place. On the contrary as storage time proceeded, the relation had the shape of a hyperbola, as can be seen in Figs. 1 and 2, and only small differences could be found among control and irradiated samples.

The sensory panel evaluations regarding the lighter colour observed in irradiated ginger and Cayenne pepper, and the unchanged colour in turmeric, agree with the chemical results. Changes in colour are not only attributable to characteristic pigment loss, but to development of browning as FARKAS (1973) points out.

Results corresponding to essential oils are reported in Table 4. Steam distillation was only carried out on ginger and Cayenne pepper; for the first one, the analysis of the non-volatile fraction (oleoresin) was also performed. No differences in the qualitative pattern were found due to irradiation, as has also been reported earlier (BACHMAN et al., 1978, JOSIMOVIC, 1983, KURUPPU et al., 1983, SAINT-LEBE et al., 1985, VAN BEEK et al., 1987). However the amount of total volatiles was in every case higher in the control samples, though, in general, without significant differences. This had been observed by us in an earlier work (NARVAIZ et al., 1990).

Table 4

Gas-chromatographic analysis of essential oils of control and irradiated ginger and Cayenne pepper, on the 60th storage day. Values represent total volatile amount, in arbitrary units (same qualitative pattern in control and irradiated samples)

Sample	Radiation dose (kGy)		
	0	10	30
Ginger (Volatile oil)	$(8.5\pm0.7)10^6$	$(7.7\pm0.3)10^6$	$(7.1\pm0.2)10^{6*}$
Ginger (Oleoresin in diethyl ether)	$(8.1\pm0.7)10^6$	$(7.9\pm0.4)10^6$	$(7.9\pm0.8)10^2$
Cayenne pepper (Volatile oil in diethyl ether)	$(18.4\pm0.7)10^6$	$(17.0\pm0.4)10^6$	$(17\pm2)10^2$

*: significantly different from control at $P \leq 0.05$ probability level; $n = 3$.

2.3. *Sensory test*

Results of samples irradiated with 30 kGy showed no significant differences from the control on pungency, flavour and odour of every sample tested during the 1st and 6th month of storage.

Colour results are reported in Table 5. It was significantly different in onion and garlic (irradiated samples became slightly darker) and in ginger and Cayenne pepper (irradiated samples became slightly lighter). In ginger this difference was not noticed in the 6th month.

Results of the sensory tests agree with the spectrophotometric colour analyses, except on onion. In onion, what is observed in the objective measurement is a loss of the typical pigments, but the darker colour observed by the sensory panel, probably due to browning reactions, did not evidence itself as an absorbance peak in the visible region.

3. Conclusions

Ground ginger, turmeric, Cayenne pepper, and onion and garlic powders were sterilized with 30 kGy.

Irradiation did not lead to significant changes in the pH of water extracts of these condiments, nor in the analyzed volatiles. Slight colour changes were observed in Cayenne pepper and ginger (lightening) and onion and garlic powders (darkening), turmeric remained inaltered. We consider this as not deleterious for the quality of these ingredients, since only turmeric is used to give colour.

Seasoning capacity was not altered with irradiation. The slight changes in colour occurred in those irradiated samples in which colour is not the typical attribute by which they are used.

*

Collaboration of panel members: KAIRIYAMA, E., NARVAIZ, P., LINDNER, C., NAKASONE, J., CHESTER, H., JUVENAL, G., KRAWIEC, L., PAGANI, M., DIAZ, A., PEDROZA, M., GABIN, M., SANCHEZ, L. and GIANGRECO, O., Dosimetry Division, Irradiation Plant of Ezeiza Atomic Center is greatfully acknowledged. Also PAGANI, M., for her assistance in performing the microbial counts, BAY, G., for her technical aid, and IDONE, C. and BARBARO, A. for their assistance.

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NUTRITIVE VALUE AND COMPOSITION OF SUGARS, TITRATABLE ACIDS AND AMINO ACIDS IN MUST OF *VITIS VINIFERA* VARIETIES

O. JUHÁSZ[a], E. DWORSCHÁK[b], É. BARNA[b]

[a]Department of Viticulture, University of Horticulture and Food Industry, H-1118, Budapest, Villányi u. 35—43. Hungary

[b]National Institute of Food Hygiene and Nutrition, H-1097, Budapest, Gyáli u. 3/a. Hungary

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Analyses of some white and red must of grapes (Chasselas, Rizlingszilváni, Olasz rizling, Hárslevelű, Táltos, Piros tramini, Fűszeres tramini, Kékfrankos, Zweigelt, Bíborkadarka, Rubintos) were carried out with samples collected in 1983 and 1984 in Szigetcsép.

A comparative study of some inorganic and organic compounds was carried out and nutritive value was characterized by indices based on the nutrient concentrations and recommended dietary allowances (RDA) among vintages of white and red grape sorts and the effect of different amounts and the application time of the nitrogen-fertilizer for musts of Tramini harvested in Balatonboglár in 1986 and 1987.

Determination of different nutrients was carried out by classical and up-to-date methods.

Significant differences were observed in the parameters among grape varieties and white and red grapes in the years 1983–84. Also significant differences were found between the effect of the treatment doses N_1 and N_2 in indices NV.

Keywords: *Vitis vinifera* varieties, nutritive value, grape must, nitrogen fertilizer

There are some concepts in the literature (DWORSCHÁK & MOLNÁR, 1978; HANSEN et al., 1979; STRMISKA, 1965) to form indices for characterizing the nutritive value of foods. We used the principle of the "Nutrient Density" for our investigations (HANSEN et al., 1979). We calculated the cumulative indices from the basic components such as titratable acids, total sugars, proteins, vitamin B group and macro- and micro elements (BENDES, 1967; STRMISKA, 1965). The must and wine are generally qualified first of all on the bases of the sugar: acid ratio and N-components, which influence the organoleptic properties as well (JUHÁSZ et al., 1987). Our aim was to extend the factors by the nutritive values (NV) for characterizing the grape.

We tried to compare the change in the NV and the mentioned components of different sorts caused by different doses and the times of application of N nutrient treatment.

1. Materials and methods

Grape samples were taken from the Experimental Station of the University of Horticulture and Food Industry in Szigetcsép in 183 and 1984 and from the Agricultural Combinate in Balatonboglár in 1986, 1987, respectively.

The experiments were carried out with must samples of Chasselas, Rizlingszilváni, Olasz rizling, Hárslevelű, Táltos, Piros tramini, Fűszeres tramini white and Kékfrankos, Zweigelt, Bíborkadarka and Rubintos red grape varieties (*Vitis vinifera* L.) and N-treated Tramini variety (Table 1).

Table 1

N-treatment of the grape variety Tramini (Tr) in Balatonboglár (BÁLÓ et al., 1988)

Code	Treatment	Amount of N-fertilizer in effective dose (kg ha^{-1})	
		1986	1987
Tr A N_1	Small dose of N-fertilizer in autumn	100	100
Tr A N_2	Increased dose N-fertilizer in autumn	200	200
Tr S N_1	Small dose of N-fertilizer in spring	0	50
Tr S N_2	Increased dose of N-fertilizer in spring	100	100

A: autumn
S: spring

For determination of nutrients the following methods were used:

— Sugar content by Fehling reaction and titratable acids (AMERINE & OUGH, 1974);

— Nitrogen content by the Kjeldahl method (BAILEY, 1967);

— Determination of vitamin B group by microbiological methods (GYÖRGY & PENSON, 1967);

— Potassium, calcium and magnesium content by flamephotometric methods (LINDNER & DWORSCHÁK, 1966);

— Essential microelements by atomic absorption spectrophotometry (PRICE, 1978).

2. Results and discussion

We calculated indices relating to the nutritive value as described in our previous paper (JUHÁSZ et al. 1987). The principle of our indices consisted in calculating the percentage of the recommended dietary allowance provided by 100 g of grape must. The mass unit was employed by STRMISKA (1965) for his index, too. For the RDA values the latest Hungarian official recom-

mendations (BIRÓ & LINDNER, 1988) were used. Indices were introduced as follows:

$$NV_{en} = \frac{(\text{sugar}) \times 15.17 + (\text{acid}) \times 10.2}{1170}$$

$$NV_{prot} = \frac{(N)}{1280}$$

$$NV_{vit} = \frac{\frac{(B_1)}{15} + \frac{(B_2)}{18} + \frac{(B_6)}{25} + \frac{(\text{Pantothenic acid})}{40}}{4}$$

$$NV_{mac} = \frac{\frac{(K)}{300} + \frac{(Ca)}{50} + \frac{(Mg)}{40}}{3}$$

$$NV_{mic} = \frac{\frac{(Fe)}{1.2} + \frac{(Zn)}{1.5} + \frac{(Cu)}{0.2} + \frac{(Mo)}{0.25} + \frac{(Mo)}{0.012}}{5}$$

We expressed the average RDA values of all nutrients in the grapes as the total nutritive value (NV). This term shows the percentage the average RDA per 100 g grape must meet with respect to the 15 nutrients examined.

$$NV = \frac{NV_{en} + NV_{prot} + NV_{vit} + NV_{mac} + NV_{mic}}{5}$$

In Tables 2–10 the data for some components and nutritive values in the grape samples are shown.

Table 2

Contents of sugar, titratable acid and vitamin B-group in grape musts
(Szigetcsép, 1983)

Samples	Sugar (g dm^{-3})	Titratable acid (g dm^{-3})	Thiamine (P_1) (μg per 100 cm^3)	Riboflavin (B_2) (μg per 100 cm^3)	Pyridoxine (B_6) (μg per 100 cm^3)	Pantothenic acid (μg per 100 cm^3)
Chasselas	190.8	6.3	16.4	3.8	58	16
Rizlingszilváni	210.0	4.2	8.4	1.0	38	35
Olasz rizling	276.5	5.2	20.0	1.2	53	37
Hárslevelű	220.6	8.5	12.5	2.6	55	24
Táltos	241.4	4.8	29.3	3.6	88	23
Piros tramini	274.8	4.3	30.0	2.9	44	75
Fűszeres tramini	248.0	3.9	20.0	2.1	37	79
Kékfrankos	210.0	7.1	27.0	1.3	38	34
Zweigelt	223.9	3.9	18.0	2.6	76	37
Bíborkadarka	234.7	6.5	21.7	3.6	65	41
Rubintos	225.8	5.8	30.0	1.5	49	31

Table 3

Contents of sugar, titratable acid and vitamin B-group in grape musts (Szigetcsép, 1984)

Samples	Sugar	Titratable acid (g dm^{-3})	Thiamine (B_1) (g dm^{-3})	Riboflavin (B_2) (μg per 100 cm^3)	Pyridoxine (B_6) (μg per 100 cm^3)	Pantothenic acid (μg per 100 cm^3)
Chasselas	27	8.1	16.0	15.4	45.3	27.0
Rizlingszilváni	97	8.1	8.1	11.3	37.6	31.0
Olasz rizling	84	11.2	17.6	9.7	62.5	41.0
Hárslevelű	198	13.3	15.2	10.2	56.4	26.3
Táltos	204	6.1	9.6	11.7	69.0	31.0
Piros tramini	255	8.2	21.3	11.8	32.7	37.0
Fűszeres tramini	208	7.2	9.2	6.2	39.0	40.0
Kékfrankos	183	9.0	11.2	13.0	58.0	29.0
Zweigelt	117	8.5	19.9	14.8	42.6	74.5
Bíborkadarka	189	10.0	14.3	15.8	46.0	102.0
Rubintos	113	11.1	15.2	20.0	53.6	44.0

Table 4

Concentrations of essential macro- and microelements in grape musts (Szigetcsép, 1983)

Samples	N	K	Ca	Mg	Fe	Zn	Cu	Mn	Mo
					(mg dm^{-3})				
Chasselas	506	833	85.0	70.0	7.3	1.58	1.24	0.66	0.030
Rizlingszilváni	465	775	102.5	75.0	2.3	0.79	2.93	0.85	0.036
Olasz rizling	513	1175	97.5	70.0	6.9	0.63	0.97	1.16	0.029
Hárslevelű	606	1625	115.0	80.0	17.3	1.05	1.93	0.90	0.051
Táltos	1136	875	102.5	80.0	1.58	0.85	2.77	0.72	0.024
Piros tramini	1013	2150	85.0	76.0	15.3	1.09	0.56	1.19	0.005
Fűszeres tramini	916	2200	75.0	90.5	13.3	1.06	0.50	1.03	0.007
Kékfrankos	782	925	100.0	80.0	8.5	0.54	2.64	0.83	0.012
Zweigelt	640	1250	100.0	71.0	3.36	1.25	1.35	0.80	0.003
Bíborkadarka	693	1542	82.5	80.5	19.3	0.61	1.00	1.36	0.038
Rubintos	694	1000	102.5	70.0	4.53	0.87	2.56	1.21	0.033

Table 5

Concentrations of essential macro- and microelements in grape musts (Szigetcsép, 1984)

Samples	N	K	Ca	Mg	Fe	Zn	Cu	Mn	Mo
					(mg dm^{-3})				
Chasselas	635	668	8.30	104	7.5	0.25	1.49	1.75	0.003
Rizlingszilváni	350	619	40.0	104	5.6	0.62	2.71	1.05	0.021
Olasz rizling	475	728	68.0	105	9.3	1.12	1.81	1.02	0.003
Hárslevelű	420	667	54.0	85	9.3	1.85	0.55	0.58	0.003
Táltos	395	489	74.0	88	7.5	1.44	2.35	0.68	0.010
Piros tramini	960	1346	45.0	96	5.6	1.09	1.35	0.82	0.010
Fűszeres tramini	1420	1905	74.0	97	10.8	0.17	1.37	1.20	0.003
Kékfrankos	605	667	95.0	101	6.8	1.30	2.23	1.34	0.003
Zweigelt	595	770	56.0	95	7.5	0.93	0.99	0.75	0.004
Bíborkadarka	660	957	53.0	104	18.9	0.76	0.65	0.79	0.003
Rubintos	920	564	69.0	92	6.0	1.42	2.46	0.94	0.028

Table 6

Total nutritive values of grape musts (Szigetcsép, 1983)

Samples	NV_{en}	NV_{prot}	NV_{vit}	NV_{mac}	NV_{mic}	Σ NV
Chasselas	1.74	0.40	1.01	2.08	3.70	1.78
Rizlingszilváni	1.89	0.36	0.75	2.17	4.70	1.97
Olasz rizling	2.48	0.40	1.11	2.54	3.62	2.03
Hárslevelű	2.03	0.47	0.94	3.24	6.52	2.64
Táltos	2.17	0.89	1.56	2.32	4.12	2.21
Piros tramini	2.45	0.79	1.45	3.59	4.29	2.51
Fűszeres tramini	2.21	0.72	1.23	3.70	3.80	2.33
Kékfrankos	1.93	0.61	1.06	2.36	4.99	2.19
Zweigelt	2.00	0.50	1.33	2.65	2.77	1.85
Bibor kadarka	2.13	0.54	1.31	2.93	6.02	2.59
Rubintos	2.04	0.54	1.20	2.38	4.95	2.22

Table 7

Total nutritive values of grape musts (Szigetcsép, 1984)

Samples	NV_{en}	NV_{prot}	NV_{vit}	NV_{mac}	NV_{mic}	Σ NV
Chasselas	0.43	0.50	1.10	2.16	4.22	1.68
Rizlingszilváni	1.37	0.27	0.86	1.82	4.92	1.85
Olasz rizling	1.23	0.37	1.31	2.14	4.38	1.88
Hárslevelű	2.77	0.33	1.12	1.81	2.86	1.87
Táltos	2.79	0.31	1.21	1.77	4.50	2.12
Piros tramini	3.50	0.75	1.08	2.60	3.25	2.33
Fűszeres tramini	2.86	1.11	0.88	3.42	4.20	2.49
Kékfrankos	2.54	0.47	1.13	2.22	4.67	2.20
Zweigelt	1.65	0.46	1.43	2.02	3.03	1.72
Bíborkadarka	2.62	0.52	1.56	2.28	4.58	2.31
Rubintos	1.61	0.72	1.34	1.85	4.87	2.08

Table 8

Content of some organic components and nutritive values (NV) in musts of grapes treated with N-fertilizer (Balatonboglár, 1986)

Parameters	Tramini			
	Spring		Autumn	
	N_1	N_2	N_1	N_2
Sugar (g dm^{-3})	190.6	180.4	206.5	203.1
Titratable acid (g dm^{-3})	5.9	5.63	5.16	5.13
Arginine (mg dm^{-3})	238.8	649.3	345.2	754.8
Proline (mg dm^{-3})	428.6	575.3	456.6	648.26
Total amino acid (mg dm^{-3})	1085.1	2197.4	1263.8	1757.56
Protein (mg dm^{-3})	240.8	507.5	481.1	—
NV_{en}	2.61	2.47	2.75	2.77
NV_{prot}	0.19	0.5	0.27	—
NV_{vit}	1.12	1.16	1.28	1.33
NV_{mac}	4.61	4.91	4.73	5.36
NV_{mic}	2.45	4.06	3.74	4.59
NV	2.19	2.62	2.57	2.83

Table 9

Content of some organic components and nutritive values (NV) in musts of grapes treated with N-fertilizer
(Balatonboglár, 1987)

Parameters	Tramini			
	Spring		Autumn	
	N_1	N_2	N_1	N_2
Sugar (g dm^{-3})	186.66	235.3	174	160
Titratable acid (g dm^{-3})	8.0	7.7	7.4	7.3
Arginine (mg dm^{-3})	500.3	610.66	707.33	603
Proline (mg dm^{-3})	173.6	183.3	159.1	150.6
Total amino acid (mg dm^{-3})	1330.8	1521.1	1615.7	1391.7
Protein (mg dm^{-3})	636.1	729.7	733.0	809.5
NV_{en}	2.49	3.11	2.32	2.14
NV_{prot}	0.403	0.76	0.506	0.73
NV_{vit}	1.04	0.903	1.25	1.42
NV_{mac}	3.96	3.76	3.91	3.903
Σ NV	1.97	2.13	1.93	2.04

Table 10

Contents of vitamin B-group in grape musts
(μg per 100 cm^3)
(Balatonboglár, 1986–1987)

Samples		Thiamine (B_1)	Riboflavin (B_2)	Pyridoxine (B_6)	Patothenic acid
Tramini					
1986					
Treatment					
Spring	N_1	21	9	44	33
	N_2	24	11	39	35
Autumn	N_1	23	13	46	43
	N_2	30	11	42	41
1987					
Treatment					
Spring	N_1	10	12	12	60
	N_2	10	10	41	30
Autumn	N_1	12	14	38	76
	N_2	13	17	40	92

The total nutritive values varied between 1.78 and 2.64 in the samples, averaging in 1983 2.21 and in 1984 2.0 for the samples of white grape varieties. In the samples of red grape varieties higher values 2.21 and 2.07 for 1983 and 1984, respectively, were observed. These data are in accord with the climatic conditions which were more favourable in 1983 than in 1984 and other years (JUHÁSZ et al., 1984) (Table 11).

Table 10/a

Concentrations of essential macro- and microelements in grape musts
(Balatonboglár, 1986–87)

Samples		Ca	Mg	Na	K	Fe	Zn
				(mg dm^{-3})			
Tramini							
Treatment:							
1986							
Spring	N_1	91.8	92.8	28.9	665.6	4.9	1.12
	N_2	83.7	91.9	27.3	610	6.6	3.84
Autumn	N_1	87.7	87.1	25.3	529.6	7.2	2.15
	N_2	94.5	90.8	31.9	663.3	9.8	1.33
Treatment:							
1987							
Spring	N_1	104.3	179.6	10.1	1596.3	—	—
	N_2	96.6	175.8	12.1	1490.3	—	—
Autumn	N_1	91.2	197.5	7.6	1495.6	—	—
	N_2	91.2	191.4	5.5	1530.3	—	—

Table 11

Growing season of cultivated grape varieties and some climatic features

Observed factors	Szigetcsép		Balatonboglár	
	1983	1984	1986	1987
Beginning of growing season	3.29	4.11	3.31	4.04
End of growing season	10.18	10.25	10.15	10.26
Duration of growing season (days)	204	198	198	206
Amount of active heat during growing season (°C)	1684	1333.5	1670	1593
Amount of effective heat during growing season (°C)	3724	3313.5	3628	3640
Rainfall during growing season (mm)	238	354	251	601
Index of precipitation-heat	4.05	4.72	4.12	9.58

Table 12/a

Significant differences among varieties in some parameters

Varieties	Sugar (g dm^{-3})	Arginine (mg dm^{-3})	Proline (mg dm^{-3})	Proteine (mg dm^{-3})	NV_{en}	NV_{vit}
Rizlingszilváni	183.96	677.26	469.73	89.42	2.16	0.95
Chasselas	159.41	763.78	136.50	147.22	1.82	0.97
Tramini	194.08	954.81	310.40	238.89	2.51	0.99
Zweigelt (kék)	187.01	479.33	586.56	122.63	2.21	1.24
Value F:	4.758	8.019	14.459	3.433	4.406	4.801

$P_{10\%} = 2.33$; $P_{5\%} = 3.01$

Table 12/b

Significant differences between white and red vine varieties in some parameters

Varieties	Years	Arginine (mg dm^{-3})	Proline	Free amino acid	NV_{en}	NV_{vit} (%)	NV
White vine varieties	1983	667.04	341.05	1894.17	1.89	1.06	1.82
	1984	581.85	214.53	2020.54	1.74	1.00	1.87
Red vine varieties	1983	626.77	977.83	2877.32	1.93	1.39	2.02
	1984	490.72	512.48	2466.68	2.76	1.21	2.08
Value F		5.817	24.447	13.425	7.876	18.370	4.571

$P_{10\%} = 2.8$; $P_{5\%} = 4.02$

Table 12/c

Significant differences in indices NV as effect of fertilizer N_1 and N_2 (1987)

Summary statistics for NV_{vit}		
Bartlett test for homogeneity of group variances		
Chi-Square = 496 DF = 1 probability	=	481
Overall mean = 1.161 standard deviation	=	0.149
Pooled within groups standard deviation	=	0.116
T statistic = 4.424 probability	=	0.000
Summary statistics for Σ NV		
Bartlett test for homogeneity of group variances		
Chi-Square = 4.649 DF = 1 probability	=	0.031
Overall mean = 2.752 standard deviation	=	0.781
Pooled within groups standard deviation	=	0.663
T statistic = 2.096 probability	=	0.045

In Tables 7–9 data of some organic components and nutritive values can be seen in must samples of Tramini in Balatonboglár treated with nitrogen fertilizer. In Tables 8–9 it can be seen that the concentration of titratable acid decreased and arginine concentration increased with treatment with dose N_2 in contrast to dose N_1.

Tables 12a–b show the significant differences in some parameters (sugar, arginine, proline NV_{en}, NV_{vit}, free amino acids) among the grape varieties.

Significant differences can be seen in the partial and total nutritive value indices of the samples treated with a lower and a higher N dose. The figures are similar in two consecutive years (Table 12c).

Red grape varieties have higher proline and free amino acid content and indices NV, lower arginine content than white grape varieties.

Among varieties the must of Tramini contains the highest sugar, arginine, proline, protein content and indices NV.

We intend to investigate the correlations between the nutritive value and the other factors in the future.

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INFLUENCE OF THE STATE OF RIPENESS OF CHARDONNAY GRAPES ON WINE COMPOSITION

II. ALCOHOLS, ALDEHYDES AND ACETOIN

M. P. CALLAO[a], J. M. BORRAS[a], A. LOPEZ[b] and F. X. RIUS[a]

[a]Departament de Quimica, Facultat de Ciències Químiques de Tarragona, Universitat de Barcelona, 43005 Tarragona. Spain

[b]Departament de Tecnologia d'Aliments, E.T.S.E.A. Universitat Politècnica de Catalunya, 25006 Lleida. Spain

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This paper illustrates the composition of alcohols, aldehydes and acetoin in wines produced from Chardonnay grapes corresponding to different states of ripeness.

Significant differences regarding the concentrations of these compounds were not found at the ripeness levels studied, which shows that advancing the time of vintage would result in wines with a low alcoholic level without a significant loss of flavour.

Keywords: Chardonnay grapes, wine composition, ripeness, alcohols in wine, acetoins in wine

One of the circumstances that undoubtedly affect the quality of a wine is the composition of the grapes used to make it. This depends, firstly, on the selected vineyard, and secondly, on the ripeness of grape. This accounts for the interest of research carried out in order to find the optimum stage of ripeness so as to obtain the maximum quality in the corresponding wine (FREGONI & IACONO, 1986). Research has been carried out about the subject using all kinds of parameters related to grape ripeness, such as sugar or potassium content, pH, the relationship between sugar and acidity, the contents of phenolic compounds, of nitrogenous substances, of aromatic compounds of the grape, etc. (ELLIS et al., 1985). In fact, knowledge of the contents of free monoterpenes, polyols and glycosides, for example, and their distribution in the skin and pulp of the grape seeds may offer a very interesting guide whether to carry out maceration or not, or to establish the most suitable conditions of pressing, so as to optimize the aroma contents of must (WILSON et al., 1986).

Notwithstanding this, the state of ripeness in the grape will determine the development of fermentation above all and, therefore, the formation of flavours originated in the course of this process. Thus, it is known, that higher alcohols are formed from the corresponding acetoacids through decarboxylation and reduction. On the other hand, acetoacids are formed either catabolically from the corresponding amino acids (Ehrlich mechanism) or synthet-

ically from carbon substances by the methods used to synthetise amino acids (McDONALD et al., 1984; HERRAIZ et al., 1989). It seems that when fermentation is faster, favoured by high temperatures and the suitable contents of solids in suspension in the must, a larger amount of higher alcohols is formed (KLINGSHIRN et al., 1987).

With regard to aldehydes, except for acetaldehyde, which is an intermediate product of the yeast metabolism (VERNIN, 1986), these are formed during the fermentation process as a consequence of the degradation of the carbohydrates that constitute the lignins (vanillin, cinnamaldehyde), or are produced during the aging process (RAPP et al., 1986). Their presence in wines at a relatively low level is probably due to the fact that during fermentation they are reduced to alcohols (RAPP & MANDERY, 1986) or oxidized into organic acids (HERRAIZ et al., 1989) or else the treatment with SO_2 changes them into hydrosoluble bisulphite combinations (VERNIN, 1986).

The apparition of acetoin in wine is produced during the fermentation of must by yeasts. Because of its condition as hydroxyacetone it is included among the compounds of carbonyl type by some authors (RAPP & MANDERY, 1986) and among the alcohols by others (VERNIN, 1986).

This paper, that complements another previously published one (CALLAO et al., 1991) shows the influence of the state of ripeness of the Chardonnay vintage on the composition of the corresponding wines in alcohols, aldehydes and acetoin. This work has been carried out at industrial level. This fact justifies the nearness of vintage dates, because it is well known that both over or under ripening have a negative effect on many parameters, such as e.g. the aroma.

1. Materials and methods

The same samples (wines from Chardonnay grapes) and analytical methods were used as those mentioned in a previous paper (CALLAO et al., 1991). The samples were frozen at −18 °C, and the examinations were carried out two or three months later.

2. Results and discussion

The chromatograms obtained from the pentane (A) and pentane/dichlorometane (B) extracts are shown in Fig. 1. The numbers in the circles above the peaks mark the alcohol as shown in the list attached to the chromatograms. The numbers in the squares designate the acetoin or the aldehydes also as shown in the identification list.

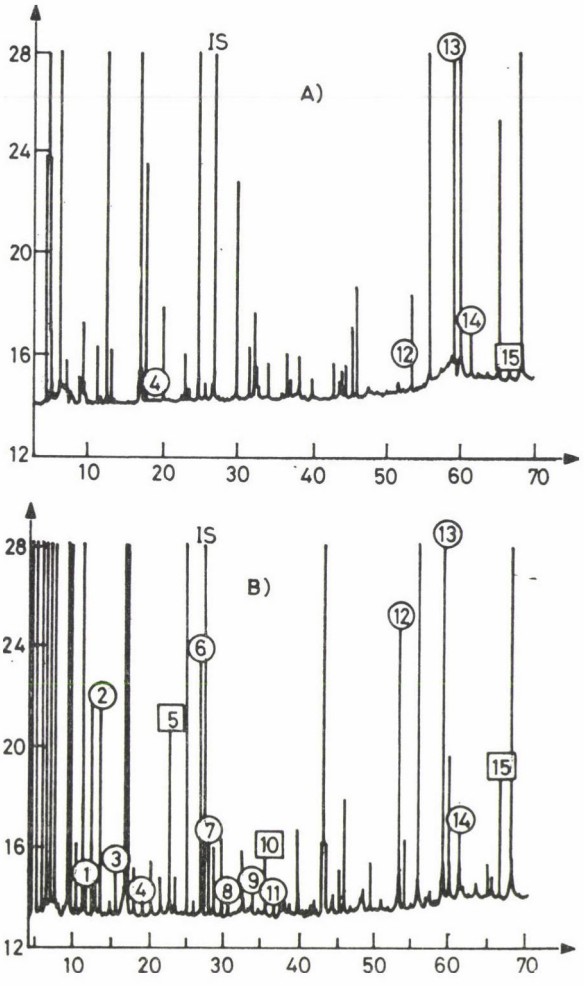

Fig. 1. Chromatograms of wine extracts: A) in pentane; B) in pentane/dichloromethane. Chromatographic conditions: Gas flow: Carrier gas, He: 0.68 cm^3 min^{-1}, air: 326 cm^3 min^{-1}; H_2: 32 cm^3 min^{-1}; auxiliary gas, He: 70 cm^3 min^{-1}, division ratio = 1/70. Working temperatures: Injector: 250 °C; Detector: 250 °C. Temperature programme: Initial: 60 °C (2 min), ramp(R): 2 °C min^{-1}, final: 180 °C (20 min)
IS Internal standard (Methyl caprilate), 1: 3-Pentanol, 2: 1-Butanol, 3: 4-Methyl-2-pentanol, 4: 1-Pentanol, 5: Acetoin, 6: trans-3-Hexen-1-ol, 7: 3-Octanol, 8: 1-Heptanol, 9: 2-Ethyl-1-hexanol, 10: Benzaldehyde, 11: 1-Octanol, 12: 1-Phenylethanol, 13: 2--Phenylethanol, 14: Benzol, 15: Cinnamaldehyde

Table 1 shows the average values of the concentrations of higher alcohols, aldehydes and acetoin eventually found in the wines, depending on the state of ripeness of the vintage used.

The evolution of each compound during fermentation, as a function of the state of ripeness in the vintage analysed, is illustrated in Fig. 2, in tri-

Table 1

Alcohols, aldehydes and acetoin in wines
(mg l^{-1})

No.	Components	Vintage			Analysis of variance	
		28-8-1986	1-9-1986	8-9-1986	F ratio	sig. level
1	3-Pentanol	0.164	0.440	0.082	138.819	0.0011
2	1-Butanol	0.152	0.002	0.166	20.646	0.0176
3	4-Methyl-2-pentanol	0.002	0.003	0.004	400.000	0.0002
4	1-Pentanol	0.109	0.147	0.209	999.999	0.0000
5	trans-3-Hexen-1-ol	0.164	0.132	0.156	5.299	0.1036
6	3-Octanol	0.027	0.040	0.017	8.472	0.0583
7	1-Heptanol	0.177	0.323	0.833	90.669	0.0021
8	2-Ethyl-1-Hexanol	0.176	0.051	0.086	0.422	0.6893
9	1-Octanol	0.259	0.321	0.262	6.525	0.0808
10	1-Phenylethanol	0.212	0.108	0.104	99.164	0.0018
11	2-Phenylethanol	4.246	6.070	10.277	5.796	0.0932
12	Benzol	0.232	0.219	0.176	999.999	0.0000
13	Acetoin	0.660	0.315	0.564	3.946	0.1446
14	Benzaldehyde	0.070	0.099	0.108	59.963	0.0043
15	Cinemaldehyde	0.450	0.382	0.196	102.354	0.0017

dimensional graphs, the numeration coinciding with that shown in the ist attached to the chromatograms.

Both Table 1 and Fig. 2 show that the overall behaviour of the compounds of alcoholic type is characterized by increasing concentration either with advancing ripeness or with advancing fermentation, or in both cases. To be more exact, 3-pentanol, 1-butanol, 1-pentanol, trans-3-hexen-1-ol, 1-heptanol, 1-octanol, 1-phenylethanol, benzol and 2-phenylethanol follow only one or both trends in a significant way (Table 1). 2-Phenylethanol, with its strong and pungent smell of roses (NYKÄNEN & SUOMALAINEN, 1988) shows the highest concentration in the wines from the ripest vintage. This is in agreement with Klingshirn's theories (KLINGSHIRN et al., 1987), who found that the higher the sugar content in the must used, the faster the fermentation (CALLAO et al., 1991).

In general, most compounds show a slight increase at the beginning of fermentation, then a sharp increase and, finally, these levels are either maintained or decrease slightly during the last stages.

Although most higher alcohols appear during the alcoholic fermentation, some of them may even appear in the must, as is the case with C_6 alcohols, in general, and other aliphatic compounds with a higher carbon content (SCHREIER, 1979). As far as this is concerned, we can confirm this trend. Thus, in spite of the low levels found, 4-methyl-2-pentanol and trans-3-hexen-1-ol have been observed in the must.

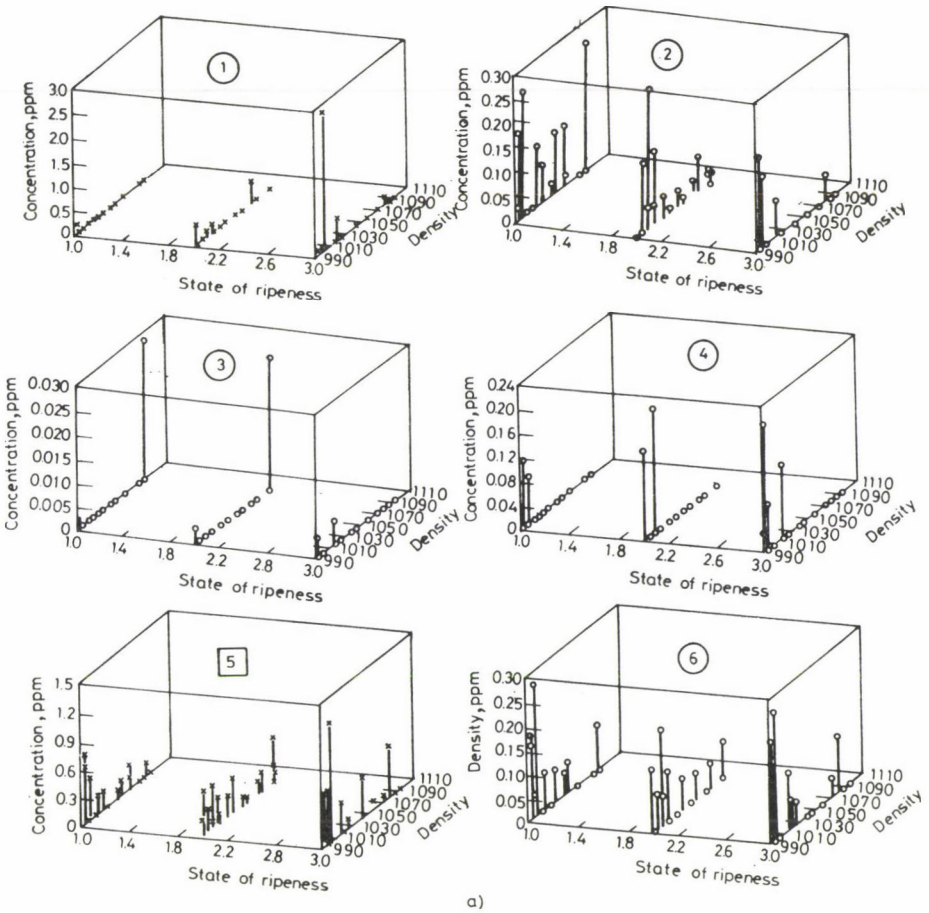

Fig. 2a. Evolution of alcohols, aldehydes and acetoin content through the fermentation peocess for each ripeness level (The concentration in wines is shown in Table 1, No. 1—6)

Regarding the aldehydes, cinnamaldehyde was the only one observed in the wine and it was found to decrease with advancing ripeness of the grapes used. On the other hand, benzaldehyde shows an opposite trend and appears to show a more or less irregular increase in concentration with advancing ripening. Except for capronaldehyde, the C_6 aldehydes in the wine were below the detection level. Capronaldehyde has been identified, but its concentration could not be established because it resolved badly in the chromatograms.

The amount of acetoin was found to increase with advancing fermentation, however finally, its concentration in the finished wine decreased, while the state of ripeness of the grapes did not produce significant differences in concentration.

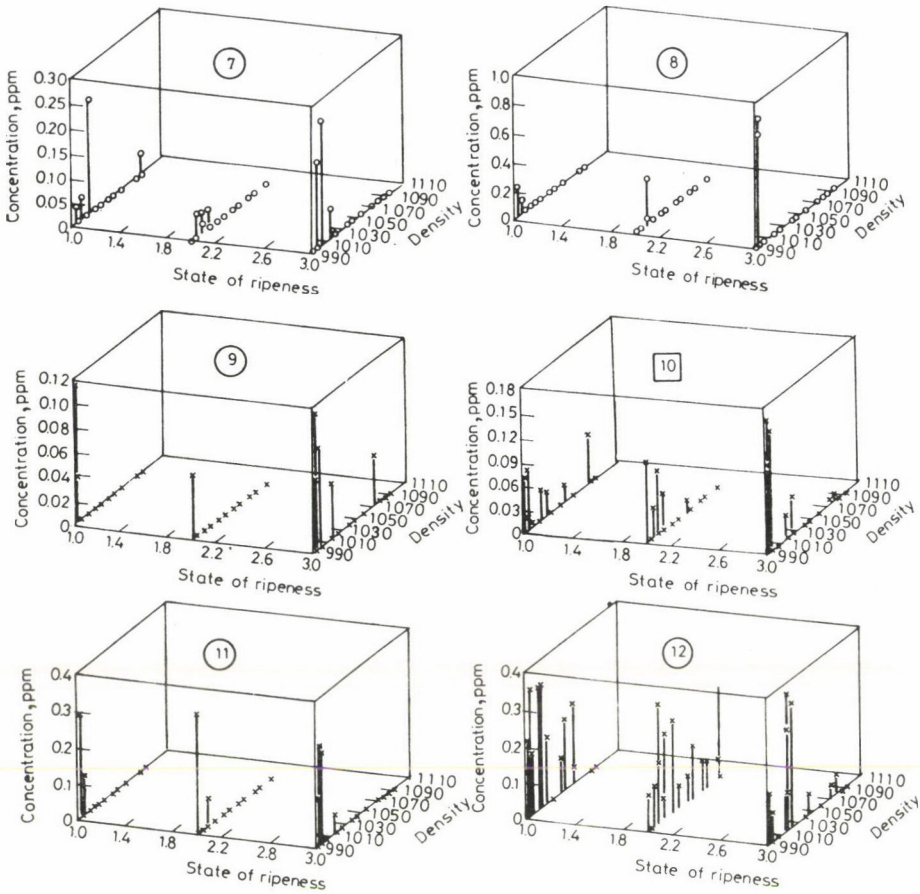

Fig. 2b. Evolution of alcohols, aldehydes and acetion content through the fermentation process for each ripeness level (Thə concentratoin in wines is shown in Table 1. No 7—12)

3. Summary

Summing up, this paper has illustrated that for most C_4 up to C_8 alcohols studied, even if their concentration rises with increasing ripeness of the grapes the increase is very slight. 1-Phenylethanol, 2-phenylethanol and benzol also show very similar levels in wines from grapes of different ripeness levels. This is also the case with benzaldehyde and cinnamaldehyde. Moreover, the different wines have not shown any significant differences regarding acetoin content. In the short period of ripeness studied, significant changes in the aroma composition were not observed, but, the conclusions put forward in the previous paper (CALLAO et al., 1991) are still valid. In other words, the

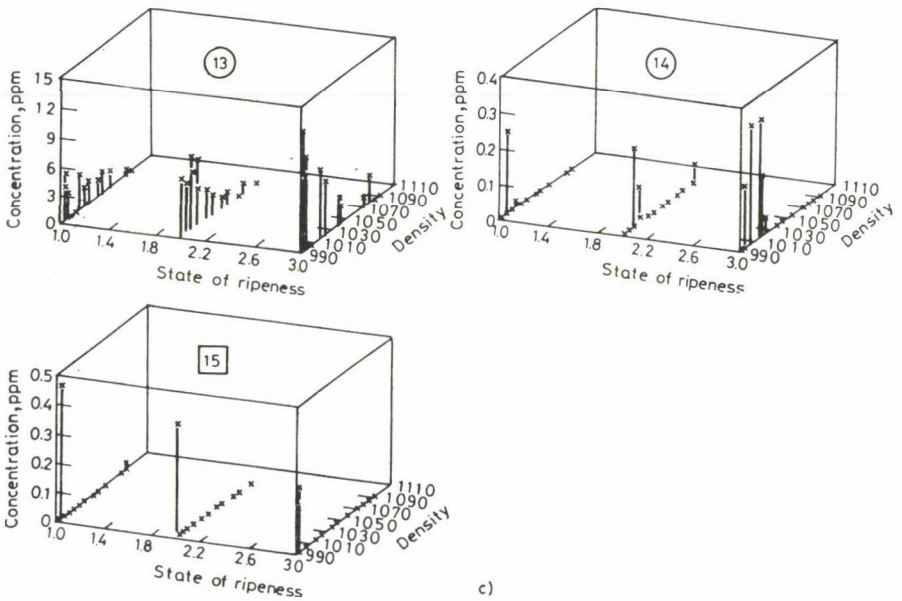

Fig. 2c. Evolution of alcohols, aldehydes and acetoin content throughout the fermentation process for each ripeness level. (The concentration in wines is shown in Table 1, No. 13-15)

vintage of Chardonnay grapes grown in this area of Spain could be carried out significantly earlier, obtaining wines of a suitable quality and relatively low alcoholic level (lower by 1.63°), without affecting the aroma contents of the wine.

*

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INFLUENCE OF THE STATE OF RIPENESS OF CHARDONNAY GRAPES ON WINE COMPOSITION

III. TERPENES AND CARBOXYLIC ACIDS

M. P. CALLAO[a], J. M. BORRAS[a], A. LOPEZ[b] and F. X. RIUS[a]

[a]Departament de Quimica. Facultat de Ciències Quimíques de Tarragona, Universitat de Barcelona, 430050 Tarragona. Spain

[b]Departament de Tecnologia d'Aliments, E.T.S.E.A. Universitat Politècnica de Catalunya, 25006 Lleida. Spain

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This paper illustrates the influence of grape ripeness on terpene and carboxylic acid composition in wines produced from Chardonnay grapes, showing that no significant differences were found in the above mentioned composition regarding the various ripeness levels. Moreover, it demonstrates the evolution of these compounds during the fermentation process, showing that, whereas all acids behave in a similar way, terpenes develop differently depending on each particular compound.

Keywords: Chardonnay grapes, wine composition, terpenes in wine, carboxylic acids in wine

Wine flavour can be classified according to the source of the different compounds that intervene in it. Thus, BAYONOVE (1987) makes a distinction between: Varietal flavour, produced by those compounds that are already present in the grapes; pre-fermentative flavours, formed by those compounds that develop during the operations of extraction and conditioning of must; fermentative flavour, due to the compounds produced by the action of yeast and bacteria during alcoholic and malolactic fermentations and, finally, post-fermentative flavour constituted by compounds that appear during the aging process through enzymatic or physico-chemical reactions.

The terpenes and their derivatives are a part of the varietal flavour, although some of them can be produced by microorganisms by biosynthesis (NYKÄNEN & SUOMALAINEN, 1988) and they lend the wine flavour a floral character.

Except for some varieties, such as muscat and malvasia, terpenic compounds are present in very low concentrations, depending mainly on the ripeness and state of health of the grape. These are present as free molecules or in combination with sugars, and they can be hydrolysed by the grape enzymes (HIDALGO, 1986). Although, in general, the concentration of terpenes increases during ripening (RAPP et al., 1978), several researchers (BOIDRON et al., 1989; USSEGLIO-TOMASSET, 1989) have pointed out that, taking into account that their level reaches a maximum value that does not coincide with the maximum

value of sugars, and decreases later, the vintage should be carried out before obtaining a maximum economic yield (industrial ripening).

However, variables such as maceration time clearly influence the levels of these compounds both in must and wine, but since a significant increase in other compounds such as polyphenols is of no interest, many efforts are being made nowadays in order to obtain the maximum yield of floral flavours with the minimum supply of other compounds that might affect the quality of wine adversely (CORDONNIER et al., 1986; BAYONOVE et al., 1984; MARAIS & VAN WYK, 1986; RAPP et al., 1984; WILSON et al., 1986; GUNATA et al., 1986).

In relation to acids, it is to be said that most of them and especially those with an even number of carbon atoms are formed by the yeast during fermentation (NYKÄNEN & SUOMALAINEN, 1988; HERRAIZ et al., 1989), that is, they form a component of fermentative flavour. No clearly defined correlation has been established between the total acid content and acid taste and it is, therefore, very difficult to verify to what extent a particular acid contributes to the acid taste. Some volatile acids affect the smell in a significant way, although their number is few because of their low volatility. SPONHOLZ and DITTRICH (1986) have found that the lower the concentration of typical fatty acids, the higher the quality of white wines.

This paper about the influence of grape ripeness on terpene and carboxylic acid composition in wines from Chardonnay grapes of the same vintage completes a series of two previous papers (CALLAO et al., 1991).

1. Materials and methods

Both the analysed samples (wines from Chardonnay grapes) and the analytical methods used are the same as those mentioned in a previous paper (CALLAO et al., 1991).

2. Results and discussion

Table 1, published in the first paper of this series, shows the most important characteristics of the original must and the derived wines. It is interesting to note that by harvesting the grapes fifteen days earlier wines with a lower alcohol content (1.63 degrees less) can be obtained.

Table 2 illustrates the concentrations of organic acids and free terpenes studied in wines from vintages of different ripeness.

Figure 1 shows the chromatograms obtained using pentane (A) and pentane/dichloromethane (B) extracts, respectively corresponding to one of

Table 1

Physicochemical characterisations of initial musts and obtained wines
(CALLAO et al., 1991)

	VINTAGE					
	28–8–1986		1–9–1986		8–9–1986	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Must						
°Brix	20.10	0.16	20.42	0.12	21.30	0.35
Titratable acidity (g l^{-1} tartaric acid)	9.86	0.12	9.60	0.06	8.16	0.11
Wines						
Alcohol content	10.55	0.10	11.42	0.12	12.18	0.05
Total polyphenol index	9.625	0.096	9.562	0.048	8.875	0.050

Table 2

Organic acids and terpenes in wine
(mg l^{-1})

	Vintage			Analysis of variance	
	28–8–1986	1–9–1986	8–9–1986	F-ratio	sig. level
Isobutyric acid	0.222	0.226	0.256	0.191	0.8357
Butyric acid	0.546	1.141	1.410	3.577	0.1606
Isovaleric acid	0.548	0.452	0.474	0.263	0.7846
Capraic acid	2.776	3.258	2.756	0.355	0.7274
Caprylic acid	5.794	6.437	4.554	0.749	0.5448
Linalool	0.112	0.076	0.040	3.743	0.1530
Terpinen-4-ol	0.188	0.142	0.122	2.261	0.2519
a-Terpineol	0.030	0.024	0.024	0.198	0.8305
Citronellol	0.086	0.083	0.188	25.795	0.0129
Nerol	0.048	0.056	0.224	6.379	0.0831
Geraniol	0.612	0.108	0.688	1.057	0.4493

the wines studied. The numbers in circles above the peaks mark the terpenes found in the extract, the squares show the number allocated to the carboxylic acids (see the legend to Fig. 1).

The evolution of the development of each compound during fermentation and regarding the different ripeness levels is shown in Figs. 2 and 3, for terpenes and carboxylic acids, respectively, as tridimensional graphs, numbered according to the information given in the chromatograms.

It can be seen in both Table 2 and in Fig. 2, that the concentrations found for the terpenes in the different wines are very low. This is due to the moderately aromatic characteristics of the Chardonnay variety and the mild conditions used in the pressing step during the must extraction; under these industrial conditions there is a very scarce contact between the grape skin and the must. Consequently, this technology, recommended in white wine

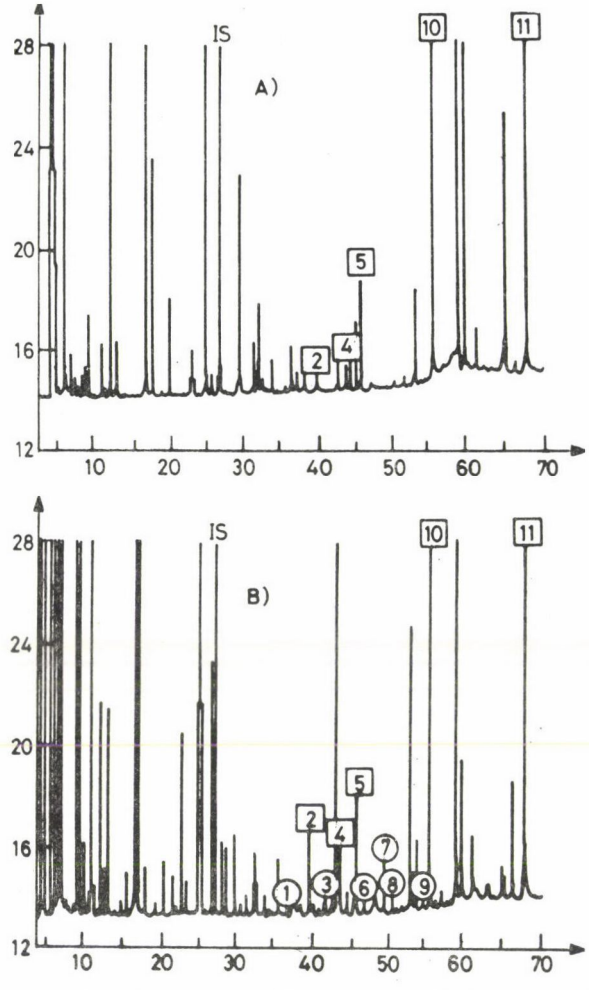

Fig. 1. Chromatograms of a wine extract. A) in pentane. B) in pentane/dichloromethane. Chromatographic conditions: Gas flow: Carrier gas, He = 0.68 cm^3 min^{-1}; air: 326 cm^3 min^{-1}; H_2 = 32 cm^3 min^{-1}; auxiliary gas, He = 70 cm^3 min^{-1}; division ratio = 1/70. Working temperatures: Injector = 250 °C; Detector = 250 °C; Temperature programme:

Initial = 60 °C (2 min); Ramp(R) = 2 °C min^{-1}; Final = 180 °C (20 min).
IS: Internal standard (Methyl caprilate); 1: Linalol; 2: Isobutyric acid; 3: Terpin-4-ol; 4: Butyric acid; 5: Isovaleric acid; 6: α-terpineol; 7: citronellol; 8: Nerol; 9: Geraniol; 10: Caproic acid; 11: Caprylic acid

vinification, induces a very low terpene extraction so that their concentration is often around the detection level of the analytical technique used. Of all the compounds geraniol is present in the largest amount. Linalool is often quoted in the literature as the most abundant monoterpene alcohol. However, in most of the cases this is referred to wines from the muscat variety and these are known to have a high terpene content. It was also observed that

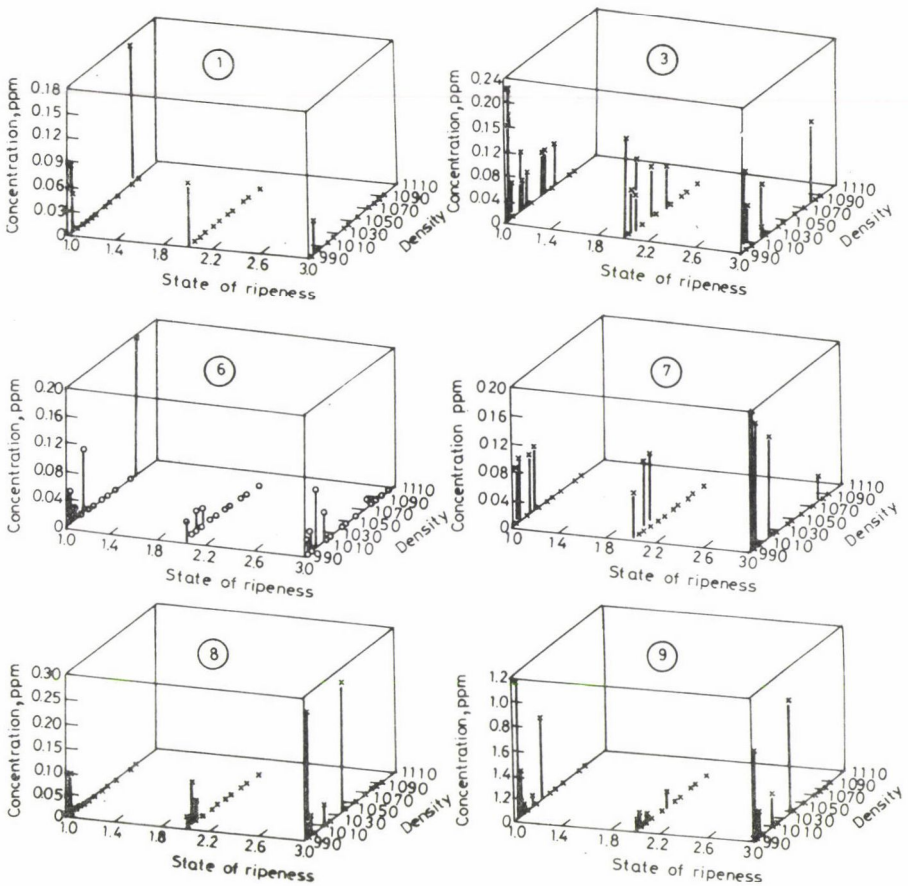

Fig. 2. Evolution of terpenes content throughout the fermentation process for each ripeness level. 1: Linalol; 3: Terpin-4-ol; 6: d-Terpineol; 7: Citronellol; 8: Nerol; 9: Geraniol. The terpenes concentration in wines is given in Table 2

there is no direct relationship between concentration and the advancement of fermentation. Thus, linalool and terpinen-4-ol appear during the initial stages of fermentation, whereas a-terpineol, citronellol, nerol and geraniol are formed only during the last stages. Different authors (Di Stefano & Ciolfi, 1983; Williams et al., 1980) have indicated an increase of the concentration level of these compounds during the fermentation process, probably due to transformations among the terpenes themselves and to the liberation of free molecules from certain compounds (Dubordieu et al., 1988). Nevertheless, in the present work, a higher content of terpenes has been detected at the end of fermentation process independently from the stage of ripeness of the grapes.

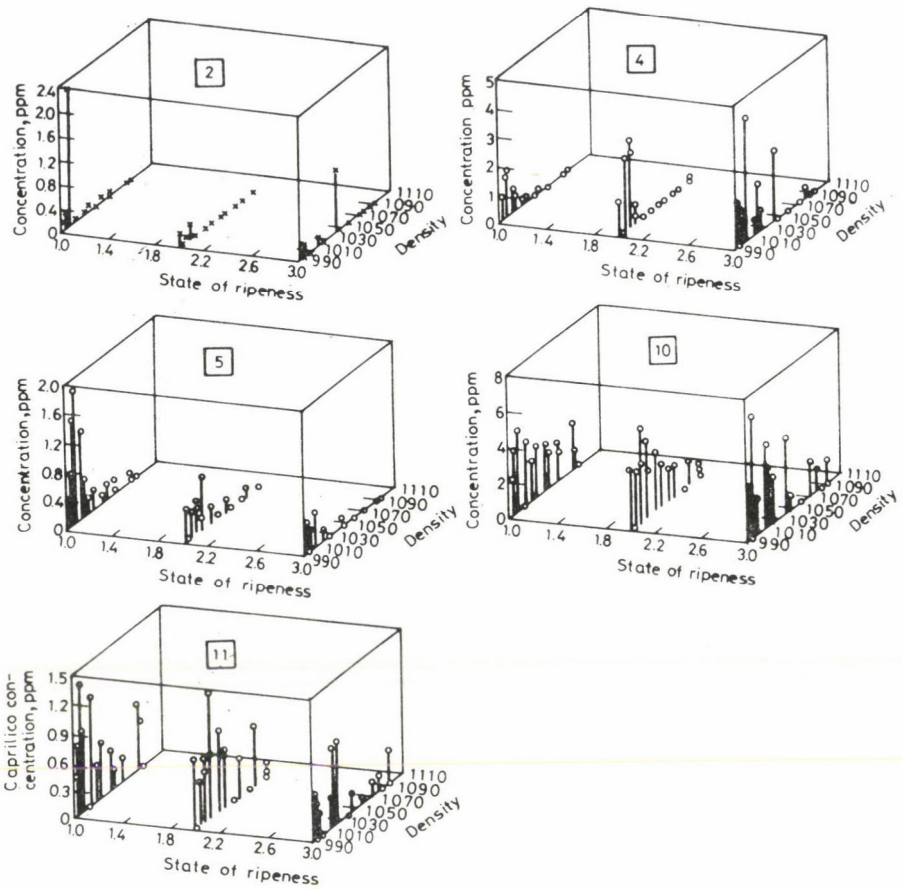

Fig. 3. Evolution of carboxylic acids content throughout the fermentation process for each ripeness level. 2: Isobutyric acid; 4: Butyric acid; 5: Isovaleric acid; 10: Caproic acid; 11: Caprylic acid. The carboxylic acid concentration in wines is presented in Table 2

With regard to the relationship with ripeness level, it can be observed that linalool and a-terpineol reach average values which are higher for the least ripe grapes, whereas for nerol and citronellol they show the opposite trend. For both terpinen-4-ol and geraniol there is no clear relationship between contents and ripeness, even apparently after comparing averages values. In any case, the differences found in the wines analysed are of little interest (Table 2). No coincident conclusions can be found in the literature about the behaviour of the individual monoterpene alcohols in relation to the state of ripeness (MARAIS, 1987; RAPP et al., 1984). However, it is agreed that each compound reaches an optimum level which may not coincide in the time scale with the highest sugar content.

The graphs in Fig. 3 and Table 2 show that the general trend regarding the acids is towards increasing in concentration as must fermentation goes on and decreasing during the last days of this process. No significant differences have been found regarding the content of organic acids in the wines studied.

No definite relationship between concentration and ripeness level has been detected. Thus, it decreases with regard to ripeness for caprylic and isovaleric acids and shows an opposed trend regarding butyric acid. Referring to isobutyric and caproic acids there is no relationship between ripeness level and concentration, even as an averages.

Some of them reach concentrations higher than 1 ppm, being caprylic acid the most abundant, followed by caproic, butyric, isovaleric and isobutyric acids, in this order. The direct application of the conclusions by SPONHOLZ and DITTRICH (1986) leads to the statement that in lack of statistically significant differences in the concentration of fatty acids in the studied wines, corresponding to vintages of different stages of ripeness, these wines did not show significant differences in quality.

3. Conclusions

On the basis of the above mentioned results it is possible to confirm the conclusions put forward in the previous paper (CALLAO et al., 1991a, 1991b), that is, the possibility of advancing the vintage of Chardonnay grapes grown in Lleida (Spain) in order to obtain wines of a lower alcohol level, since no significant differences have been found (P = 99%) between the organic acid and terpene content in the different wines studied, due to using grapes in different stages of ripeness, under the conditions applied in this study.

*

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DEVELOPMENT OF METHODS AND AN EXAMPLE FOR THE DETERMINATION OF KINETIC CONSTANTS IN RELATION TO THE HEAT TREATMENT OF FOOD

I. KÖRMENDY

Institute of Food Technology, University of Horticulture and Food Industry, H-1118 Budapest, Ménesi út 45. Hungary

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Heat treatment of food results in changes of quality attributes (concentration of vitamins, sensory attributes, activity of enzymes, number of surviving microbes, etc.). As the kinetic treatment of the variation of food quality attributes is mostly incomplete at present, the author proposes to regard the initial value of the quality attribute and the homogeneity of the dispersions, when employing the empirical kinetic equations (adopted from chemical engineering) for evaluating kinetic constants from experimental data. Moreover, the author made significant amendments on evaluation methods and presented an example. He recalculated the results of HORAK and KESSLER (1981), who had investigated the decrease of thiamin content in milk, and compared three different evaluation methods. Method 1 is based on independent regression analyses at different temperatures and interpolation between subsequent temperatures according to the Arrhenius-equation. Method 2 uses the deficient process of two consecutive regression analyses (it has been often employed in the past). Method 3 is based on the concept of least squares and here all kinetic constants are evaluated from a system of equations. The application of Method 2 resulted in unexceptably great confidence intervals for the energy of activation ($E_a = 90.34 \pm 50.06$ kJ mol^{-1}) in contrast to Method 3 ($E_a = 81.50 \pm 7.32$ kJ mol^{-1}), therefore Method 2 should be avoided in the future. Method 1 has been found the most appropriate for the prediction of a thiamin concentration to previously selected temperature and time ($120\ °C \leq T \leq 150\ °C$, $0 < \tau \leq 5000$ s), as the mean variance of fitting is 1/5 to 1/4 part of the respective values from Methods 2 and 3. Namely the variance of predicted values is proportional to the previously mentioned variances.

Keywords: food, heat treatment, variation of quality attribute, kinetic constant

The rate of variation of a quality attribute[a] of a food is highly dependent on the temperature. The degree of dependency is mostly stated as the energy of activation (E_a) or the z-value (HEISS & EICHNER, 1984). The quality attributes are of various nature: Compositional attributes include the concentration of components (e.g. of vitamins), of surviving microbes; the activity of enzymes. Sensory attributes are scored by a panel of experts and the result can be comprehended as the measured value for such type of scoring, where mean and standard deviation are obtained. Physical properties (e.g. objective colour, rheological constants) are also treated as quality attributes. Examples

[a] In a previous article (KÖRMENDY, 1987) the unusual term "property value" has been applied instead of "quality attribute".

for quality attributes: concentration of ascorbic acid in paprika concentrate, concentration of thiamin in canned meat product, consistency of canned peas as measured by a shear press or tenderometer, number of surviving active spores.

This publication treats such experimental arrangements, where the value of a quality attribute, starting from some initial value, is being measured at different time intervals, other conditions having been fixed. A systematically varied quantity, which contributes to the fixed conditions, is named the parameter of the variation of the attribute. Such parameters are temperature, water activity, concentration of a preservative, intensity of irradiation.

Here, regarding the heat treatment of food, only one parameter, the temperature is involved, and the relation between the measured quality attribute (A) and time (τ) can be illustrated by a number of curves. Each curve belongs to a selected temperature (T) and is obtained by some fitting procedure. In many cases the relations of the empirical reaction kinetics (LEVENSPIEL, 1972) are appropriate for this task.

Some reasons for the adoption of the empirical kinetic equations are as follows:

— Quality attributes include the concentration of selected components in food.

— The equations have been already used in food science: the equations of first order reactions often, those of zero or second order have been less frequently fitted to measured values (LABUZA & KAMMAN, 1983; DANNENBERG & KESSLER, 1986; TAOUKIS & LABUZA, 1989).

— The equations are of such type, that the quality attribute depends on the product of time and a constant at fixed temperature. The constant (which is the rate constant of variation here) depends only from the temperature. It is possible in such cases to find a proper transformation for the quality attribute, so that the transformed quality attribute shows a linear relation vs. time at constant temperature. It has been also demonstrated for equations of this nature, that *in case of time dependent temperature* the variation of a quality attribute can be calculated through the intermediary step of calculating first the equivalent heat treatment time (KÖRMENDY, 1982, 1987; TAOUKIS & LABUZA, 1989).

The aim of the author was to develop and increase the exactness of methods used for the determination of kinetic constants belonging to the variation of quality attributes. His results are based on the method of least squares (MANDEL, 1964) and he thought it necessary to underline the importance of the examination of the homogeneity of variances, as well as of the influence of the initial value of a quality attribute on variation. It has been already recognized that the determination of the energy of activation from two subsequent regression analyses is far from being exact (ARABSHAHI &

LUND, 1985; SAGUY & KAREL, 1987). The author has recalculated the data of HORAK and KESSLER (1981) and drew conclusions, which are apt for generalization.

1. Theoretical considerations

If the quality attribute (A) is measured at preset temperatures (T) and times (τ), then the fluctuations in temperature and time increase the dispersion of the quality attribute. The classification of experimental and evaluation methods can be made expediently first according to the initial value of the quality attribute (A_i): various initial values exist at different temperatures; one common but not preset initial value exists; the initial value is preset. In the last case the existing variance of the initial value also increases that of the quality attribute and hence the variances of the different calculated kinetic constants. Therefore the initial value here can be regarded as second parameter of variation in addition to the temperature.

Different evaluation methods belong to the previously mentioned types of initial values (see Table 1). There is a function of maximum three independent variables (τ, T, A_i; the dependent variable being the quality attribute) to be fitted to measured values. The kinetic constants are to be determined, so as the sum of squares of differences between measured and calculated quality attributes and weighed according to the reciprocals of variances should have the smallest value (the method of least squares, HALD, 1962; MANDEL, 1964).

According to the author's idea, at present such version of the least squares method should be applied where the sum of squares of the transformed quality attribute is minimized.

The idea is substantiated by the fact, that the generalized concepts of rate constant and time constant are connected to the transformed quality attribute. Probably results produced with the sum of squares regarding the original attribute will somewhat differ from that obtained by the previous method. It is advisable to analyse this question in the future. Thus the author's method provided results for the transformed attribute. From the estimated expectation value and dispersion of the transformed attribute one can calculate those of the original attribute, at least, if the conditional distributions of the transformed attribute around the regression line are homogeneous and normal. As a result of former considerations the initial concentrations (A_i) in Tables 3, 5 and 6 are only approximatively precise.

In this publications only such variations are considered, which can be described by the differential equation:

$$\frac{dA}{d\tau} = -k_n(T)(A - A_0)^n \qquad (1)$$

In addition to already known symbols A_0 is a constant and the difference between the quality attribute and this constant is characteristic for the variation. $k_n(T)$ is the rate constant depending on the temperature. n is the dimensionless exponent of the order of variation, analogous to the order of reaction ($-\infty < n < +\infty$).

Solution of eqn 1 at constant temperature and with the initial condition $\tau = 0$, $A = A_i$ results in the following equations:

If $n = 1$, then

$$U = \lg \frac{A - A_0}{A_i - A_0} = -\frac{k_1 \cdot \tau}{\ln 10} \tag{2}$$

If $n \neq 1$

$$U = \left(\frac{A - A_0}{A_i - A_0}\right)^{1-n} = 1 - \frac{(1-n)k_n}{(A_i - A_0)^{1-n}} \tau \tag{3}$$

The quantity U in the equations can be called the dimensionless transformed quality attribute and is a linear function of time at constant temperature. The original quality attribute presents linear dependence on time only in the case of zero order change. Other linear transformations of U (e.g. $\lg (A - A_0)$, $(A - A_0)^{1-n}$) depend also in a linear way on time. However these transformed attributes are not necessarily dimensionless (e.g. $[A]^{1-n}$). The concept of "quality function" (TAOUKIS & LABUZA, 1989) is essentially equivalent to the transformed quality attribute. In case they are unknown, it is necessary to find by systematic variation those values of A_0 and n, which values can be regarded as providing the highest linearity for the variation of the transformed attribute.

After selecting the appropriate transformation (the values of A_0 and n), the dispersions of the transformed attribute around the regression lines should be tested for homogeneity (e.g. with the help of Bartlett's chi-square test). If the dispersions' homogeneity is accepted, i.e. the dispersions show only casual fluctuations, then according to the author's terminology a *regular* case is faced. It is known that in such case the weighing of squares of differences can be omitted in the regression analysis. The proper version of the regression analysis can be selected from Table 1. The dual of the serial numbers of the row and column assign a variant component in the table. The totality of different variant components represent a selected variant.

The numbers before and after the point in Table 1 indicate the serial number of row and column, respectively. The sign + means the symbolic addition of the components (the AND in logic). The table does not include such variants, which treat experiments with parallelly measured data.

For example the meaning of variant (1.1 + 2.1 + 3.1 + 4.1) is the following: Different initial values belong to each temperature, the rate constants are independent of the initial value, the rate constant depends on temperature

Table 1

Components of the versions of regression analyses for the determination of kinetic constants

Serial number of category	The aspect of a category	Division of a category to components		
		1	2	3
1	Type of the initial value of the quality attribute (A_i)	1.1.: Various initial values at different temperatures	1.2.: Previously not known common initial value	Previously set common initial value
2	Relation of the rate constant (k_n) and time-constant (D_n) to the initial value	2.1.: k_n is independent of A_i	2.2.: D_n is independent of A_i	—
3	Dependence of the kinetic constants on temperature	3.1.: The Arrhenius equation is supposed to be valid (E_a = constant)	3.2.: It is supposed that z = const. (see eqn 7)	—
4	Domain of temperature for kinetic constants	4.1.: constants are valid in the total range of temperature	4.2.: Constants have different values in subsequent intervals of set temperatures	—

according to the Arrhenius equation, the single energy of activation is valid between the highest and lowest set temperatures. Further explanations to Table 1: One component in a row excludes the selection of another in the same row; if $n = 1$, then both the rate constant and time constant are independent of the initial value of the quality attribute.

The time constant, according to the authors proposal, is the reciprocal of the factor before the time in eqn 2 and eqn 3, i.e. if $n = 1$, then

$$D_1 = \frac{\ln 10}{k_1} \tag{4}$$

if $n \neq 1$

$$D_n = \frac{(A_i - A_0)^{1-n}}{(1 - n)k_n} \tag{5}$$

If $n = 1$, the absolute values of the time constant is the generally known decimal reduction ($D_1 > 0$) or decimal multiplication ($D_1 < 0$) time. Dimension of D_1, D_n is the dimension of time, i.e. *s* or min.

If $n \neq 1$, the time constant depends also on the initial value of the quality attribute.

The absolute value of the time constant is the time needed for unit variation of the absolut value of U. k_n and D_n both might have positive and negative values. If $D_n < 0$ U increases in time, otherwise ($D_n > 0$), decreases (see eqn 2 and eqn 3 after placing D_1 and D_n into them with the help of eqn 4 and eqn 5).

The relation between the rate constant and the initial value of the quality attribute deserves special attention. As a consequence of the empirical character of kinetics, the relation can be determined in most cases experimentally. Letone consider, as an explanation, the instance already recognized by ARABSHAHI and LUND (1985):

If a first order variation (at constant temperature) is approximated by zero order changes, then the zero order time constants belonging to various initial values of the attribute will be equal, while the zero order rate constants will be proportional to the initial value according to the relation, $k_0 = (A_i - A_0)D_0$. Further explanation is given in Fig. 1.

Thus from small changes with different initial values one will conclude that the time constant (and not the rate constant) is independent of the initial value of the quality attribute. In the same way, if k_2 of a second order variation is independent of the initial value, then D_0 will be proportional to the reciprocal of $(A_i - A_0)$, while k_0 to the square of $(A_i - A_0)$.

If k_n or D_n depends on the initial value, then a *reference initial value* also should be stated to relate their values at different temperatures to a common basis.

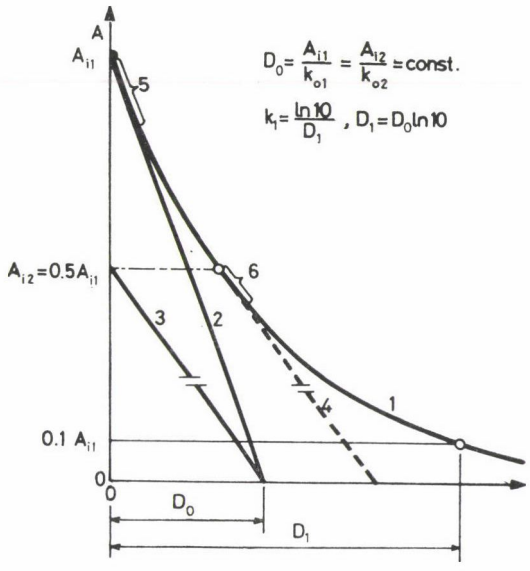

Fig. 1. Approximation of the sections a first order variation by zero order changes. 1: first order variation; 2, 3: zero order changes approximating the initial and middle sections of the first order variation; 4: tangent to the curve of the first order variation, passing parallel with line 3; 5, 6: sections of acceptable approximation. Dotted line: direction of shifting the tangent. D_0, D_1, time constants of zero and first order variations, respectively

The 3rd row of Table 1 includes (already known) relations between the rate constant or time constant and the temperature. The expedient form of the Arrhenius relation is

$$\frac{k}{k_r} = \frac{D_r}{D} = \exp\left[-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_r}\right)\right] \tag{6}$$

while for the version z = constant

$$\frac{k}{k_r} = \frac{D_r}{D} = 10^{(T-T_r)z} \tag{7}$$

The subindex n has been omitted in the notations of the rate constant and time constant, subindex r relates to the reference temperature (T_r), while notations without subindex to temperature T. The energy of activation is denoted with E_a, the universal gas constant with R, z is the temperature increment to achieve a tenfold increase of the rate constant. The same initial value of the quality attribute belongs to all rate constants and time constants. Only absolute temperatures can be introduced into eqn 6.

Variant component 4.1. means that only a single k_r or D_r, E_a or z is evaluated in the total range between the highest and lowest temperatures. However, different values of the previously mentioned kinetic constants in subsequent temperature domains of set temperatures are received by applying the variant component 4.2. These can be regarded as interpolation constants. Para. 2 and para. 3 give more explanation.

Methods applied in the example (para. 2) are based on the following principles: First A_0 and n are to be determined (including tests for linearity).

Next other kinetic constants are evaluated: The sum of squares of differences between transformed values of measured attributes and calculated ones (S) are formed. The proper values of kinetic constants are those, which minimize S. The minimum of S can be found by its differentiation partially by the kinetic constants, making the differential quotients equal to zero and solving the system of equations thus formed (there are as many equations as the number of kinetic constants). The segregation of the determination of A_0 and n from that of the other kinetic constants is a simplification. It would be more exact to include their determination in the process of finding the minimum of S. Omission of weighing by the reciprocals of variances from the sum of squares is justified only if variances are homogeneous.

2. Example

The example is based on the publication of HORAK and KESSLER (1981), who measured the decrease of thiamin concentration in whole milk. They applied heat treatment at temperatures 120, 130, 140, 150 °C and times from 20 *s* to 5000 *s*. Data can be seen in Table 2.

Parallel experiments had not been mentioned and the author here presumed that a *regular* case is faced (see in para. 1) and adopted the original results that the concentration (A) changes according to the second order ($n = 2$) and approximates zero in time (i.e. $A_0 = 0$).

Substitution of $A_0 = 0$ and $n = 2$ in eqn 3 results in

$$y_j = y_{ij} + k_{2j}\tau \tag{8}$$

where $y_j = 1/A_j$ and $y_{ij} = 1/A_{ij}$. Symbol j is the serial number of temperatures ($1 \leq j \leq J = 4$). The values of y_{ij}, k_{2j} and other kinetic constants will be determined by applying different versions of regression analysis. The reciprocals (y_{jl}) of measured concentrations (A_{jl}) have been introduced into the calculations, where subindex l is the serial number of measured values at temperature with serial number j. The measured initial values will also differ from the calculated ones. In the subsequent treatment symbols k and D will be applied instead of k_2 and D_2.

Table 2

Time dependence of the concentration of thiamin in milk at various temperatures

Time (s)	Concentration, A (mg dm^{-3})			
	120	130	140	150
0	0.32	0.31	0.39	0.39
20	—	0.31	—	0.38
50	0.32	0.31	0.36	0.35
100	0.32	0.28	0.34	0.28
200	0.31	0.28	—	—
300	—	—	0.29	0.20
400	0.27	—	—	—
500	—	0.23	—	—
600	—	—	0.21	0.15
1000	0.23	0.19	0.15	0.10
1500	—	—	0.12	—
2000	—	0.13	0.10	—
3000	0.16	—	0.07	—
5000	0.13	0.06	—	—

All confidence limits are related to the probability level of 5 percent in Table 3 to Table 6.

The different variants of evaluation are as follows:

Method 1 is the variant (1.1 + 2.1 + 3.1 + 4.2) in Table 1. Independent analyses of regression at set temperatures served for the determination of A_i, k and D (see Fig. 2 and Table 3). The confidence limits of the reciprocal of A_i and k can also be seen in Table 3.

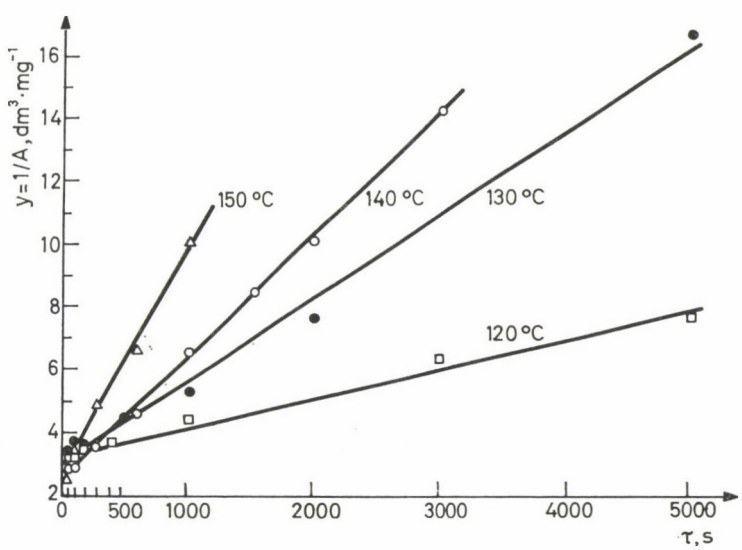

Fig. 2. The reciprocals (y) of measured thiamin concentrations (A) vs. time (τ) and the regression lines according to Table 3

Table 3

Results of regression analyses of data in Table 2. The results have been used in *Method 1 and 2.* The regression lines represent relations: $y = (1/A = (1/A_i) + k\tau$ (subindex j has been omitted here)

Temperature (°C)	Initial value of concentration, A_i (mg dm^{-3})	Reciprocal of the A_i and confidence limits (dm^3 mg^{-1})	Rate constant and confidence limits (dm^3 g^{-1} s^{-1})	Time constant D[a] (min)	Variance of fitting[b] s^2 (dm^3 mg^{-1})^2	Coefficient of correlation	Degrees of freedom
120	0.3149	3.1754 ± 0.2068	$0.9433 \times 10^{-3} \pm 9.85 \times 10^{-5}$	−56.14	0.0379	0.9946	6
130	0.3311	3.0198 ± 0.3287	$2.6617 \times 10^{-3} \pm 17.92 \times 10^{-5}$	−18.91	0.1237	0.9972	7
140	0.3987	2.5084 ± 0.2014	$3.8833 \times 10^{-3} \pm 14.78 \times 10^{-5}$	−10.77	0.0335	0.9991	7
150	0.3849	2.5980 ± 0.2771	$7.2967 \times 10^{-3} \pm 60.62 \times 10^{-5}$	− 5.93	0.04730	0.9974	5
				Mean ($\overline{s^2}$):	0.0626	—	25

[a] $D = -\frac{1}{kA_i}$

[b] Sum of squares of differences between reciprocals of measured and calculated concentrations divided by the degrees of freedom

Table 4

Energies of activation as calculated from rate constants in Table 3, in subsequent domains of set temperatures
(Method 1, T_r = 121,11 . . . °C)

Domain of temperature (°C)	Energy of activation (E_a) and confidence limits (kJ mol^{-1})	Rate constant at the reference temperature (T_r) k_r (dm^3 mg s^{-1})
120–130	136.69±14.6	1.0617×10^{-3}
130–140	52.31± 9.71	1.8717×10^{-3}
140–150	91.68±11.44	1.0817×10^{-3}

In this version, as presented in Table 4, three energies of activation and three rate constants at the reference temperature have been evaluated according to three domains between set temperatures. It means that the rate constant in between subsequent set temperatures is now calculated by interpolation procedure using the Arrhenius equation (see Fig. 3). One might consider an assumption, too, that E_a and k_r have some kind of temperature dependence.

The confidence limits in Table 4 have been calculated from the estimated dispersions of the activation energies ($s(E_a)$) from the relation

$$s(E_a) = \frac{R}{T_j^{-1} - T_{j+1}^{-1}} \left[\frac{s^2(k_j)}{k_j^2} + \frac{s^2(k_{j+1})}{k_{j+1}^2} \right]^{1/2} \tag{9}$$

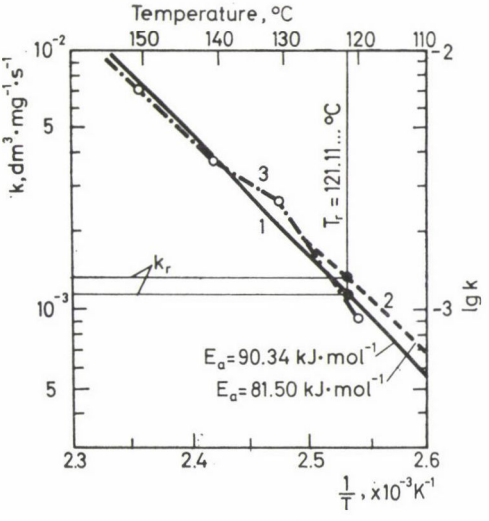

Fig. 3. Relations between the rate constant and the temperature, based on results in Tables 4, 5 and 6. 1: regression line according to Method 2; 2: line pertaining to the value of E_a and k_r obtained by Method 3; 3: interpolation lines connecting the points (lg k, $1/T$) as obtained by Method 1. Full circles (●) represent values of k_r from Method 2 and 3

The dispersion of k in the above relation ($s(k)$) is one of the already known results of the regression analysis (VOLK, 1969). The degrees of freedom of $s(k_j)$ and $s(k_{j+1})$ were added for the calculation of the confidence limits of E_a. The rate constant at a temperature between T_j and T_{j+1} can be calculated by a simple interpolation formula, deducible from eqn 6. Eqn 9 has been deduced on the law for calculating the dispersion of the function of a probability variables.

Method 2 is the variant (1.1 + 2.1 + 3.1 + 4.1) in Table 1, however, *two subsequent regression analyses* are applied. Here the logarithms of the rate constants calculated by Method 1 and the reciprocals of the absolute temperatures have been treated by a second regression analysis and E_a, k_r were evaluated from this second analysis. In this variant the principal regression lines of Method 1 (at constant temperatures) are modified by the new rate constants (k'), which are positioned on the regression line of the second analysis and new initial values (y'_i, A'_i) belong to the modified relations. Results are presented in Table 5.

In this version the confidence limits of E_a have been received from the regression analysis between $\ln k$ and $1/T$, as an already well known result, the degrees of freedom being: $J - 2 = 4 - 2 = 2$ (!).

Method 3 is the same variant as Method 2 (variant (1.1 + 2.1 + 3.1 + + 4.1), but for applying one common regression analysis (or common least squares method). In this variant the author formed the total sum of squares of differences between the reciprocals of measured concentrates and respec-

Table 5

Results pertaining to Method 2

Relation $\ln k' = \ln k_r - \frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_r}\right)$ has been fitted to the rate constants of Table 3 (independent variable: $1/T$, dependent one: $\ln k'$, $T_r = 121{,}11\ldots$ °C)

Temperature (°C)	initial value A'_i (mg dm^{-3})	Reciprocal of A'_i (dm^3 mg^{-1})	Modified rate constant k' (dm^3 mg^{-1} s^{-1})	Mean variance of fitting, s^2_{ia} (dm^3 mg^{-1})^2	Degrees of freedom
120	0.3314	3.0171	1.0733×10^{-3}		
130	0.2821	3.5447	2.1300×10^{-3}	0.3157	27
140	0.4324	2.3126	4.0900×10^{-3}		
150	0.3994	2.5039	7.6150×10^{-3}		

Energy of activation (E_a) and confidence limits: $90{\cdot}34 \pm 50.06$ kJ mol^{-1}
Rate constant at the reference temperature: k_r: $1{\cdot}16.10^{-3}$ dm^3 mg^{-1} s^{-1}
Variance of fitting: 0.0353
Degrees of freedom: 2
Coefficient of correlation: -0.9832

[a] the concept is explained in Table 3

Table 6

Results pertaining to Method 3

The linear relation $y = 1/A = (1/A_i) + k_r \exp[-(E_a/R)(1/T - 1/T_r)] \cdot \tau$ has been fitted to the reciprocals of concentrations in Table 2 (independent variable: τ dependent variable y, $T_r = 121{,}11 \ldots$ °C).

Temperature (°C)	Initial value A_i (mg dm^{-3})	Reciprocal of A_i (dm^3 mg^{-1})	Rate constant, k (dm^3 mg^{-1} s^{-1})	Mean variance of fitting, $s_0^2(y)$ (dm^3 mg^{-1})^2	Degrees of freedom
120	0.3556	2.8124	1.2400×10^{-3}		
130	0.2964	3.3741	2.3033×10^{-3}	0.2600	27
140	0.4430	2.2574	4.1483×10^{-3}		
150	0.3836	2.6071	7.2667×10^{-3}		

Energy of activation and confidence limits: $81{\cdot}50 \pm 7{\cdot}32$ kJ mol^{-1}
Rate constant at the reference temperature, k_r: $1{\cdot}3317.10^{-3}$ dm^3 mg^{-1} s^{-1}

tive calculated reciprocals, including all temperatures. He built into this term the Arrhenius equation and searched for the kinetic constants (A_{ij}, E_a, k_r). Thus the kinetic constants are evaluated by substitution for k in eqn 8 the right side of eqn 6 multiplied by k_r, next forming the total sum of squares (S) including all temperatures. Differentiation of S partially by y_{ij}, k_r and E_a conduces to the system of equations:

$$\frac{\partial S}{\partial y_{ij}} = 0, \ (j = 1, 2, 3, 4), \frac{\partial S}{\partial k_r} = 0, \frac{\partial S}{\partial E_a} = 0 \tag{10}$$

It is possible to obtain from eqns 10 k_r and y_{ij} in explicit formulae, if the value of E_a is previously known, however, E_a can not be explicitly expressed. Therefore the author has changed the value of E_a step-wise, decreased the step intervals as far as finding that value of E_a, then of k_r, then of four values of y_{ij} to which values the minimum of S belongs (see the end part of para. 1).

Results are presented in Table 6, including the confidence limits for E_a.

The author obtained the confidence limits of E_a from its estimated variance, the latter having been calculated with the formula

$$s^2(E_a) = R^2 \frac{\sum_1^J \left[\sum_1 (\tau_{jl} - \bar{\tau}_j)^2 \right]^{-1}}{\sum_1^J \left[k_j(\tau_j^{-1} - \tau_r^{-1}) \right]^2} s^2(y) \tag{11}$$

The summation of the square of $\tau_{jl} - \bar{\tau}_j$ is made at a temperature with serial number j according to serial number $l \cdot s^2(y)$ is the mean variance of fitting as presented in Table 6. Eqnation 11 was derived by the author on the assumption,

that the variance of the rate constant depends only on the variance of the energy of activation, that is, fluctuations in the rate constant were attributed to fluctuations in the energy of activation (consequently the variance of k_r has been also neglected).

Regarding the law to calculate the dispersion of a function of a probability variable, it is possible to obtain at temperatures denoted by T_j the following relations:

$$s^2(k_j) = s^2(y) \sum_2 (\tau_{j1} - \bar{\tau}_j)^2 = s^2(E_a) \; [k_j(T_j^{-1} - T_r^{-1})]^2 R^2.$$

The summation of these relations (which means at the same time the formation of the mean of $s^2(k_j)$ results in eqn 11.

3. Results and discussion

Method 1 produced three paired values of E_a and k_r. These can be regarded as constants of interpolation for the calculation of the rate constant between set temperatures. However, the interpolation can be executed by the (direct) formula, too:

$$\ln k = \ln k_j + \frac{T^{-1} - T_j^{-1}}{T_{j+1}^{-1} - T_j^{-1}} (\ln k_{j+1} - \ln k_j)$$

It also can be proved that the variance of k stands between the variances of k_j and k_{j+1}.

The defective procedure of Method 2 gave a single energy of activation with erroneous mean and extremely high confidence levels. The difference between the energy of activation of Method 2 and the correct Method 3 was about 10 percent and the confidence limits in Method 3 decreased to ± 7.32 kJ mol^{-1} from ± 50.06 kJ mol^{-1} of Method 2. It is Method 3 to be recommended for the determination of the energy of activation instead of the formerly often used Method 2, if one is interested in the energy of activation in the total range of set temperatures. The difference between the values of k_r from the two methods was yet higher, about 14 percent (see Table 5 and Table 6).

However, if one wants to predict (to set temperature and time) the value of a quality attribute, i.e. the thiamin concentration is this case, then Method 1 seems to be the most appropriate. The mean variance around the regression lines is considerably lower in this version than in the other two methods:

In Method 1 $s^2 = 0.0626$ dm^6 mg^{-2} (see Table 3), in Method 2 $s'^2 = 0.315$ dm^6 mg^{-2} (see Table 5) and in Method 3 $s^2(y) = 0.260$ dm^6 mg^{-2} (see Table 6).

It is well known that the variance of that variable which is estimated from the regressional relation (dependent variable) is proportional to the previously mentioned mean variance.

The final conclusion is that the energy of activation in the total domain of temperature should be determined by Method 3, while Method 1 is the most suitable to predict a value of a quality attribute.

*

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EXPERIENCE WITH NUTRITION POLICY IN EUROPE[a]

E. HELSING

WHO Regional Office for Europe, DK-2100, 8, Scherfigsvei, Copenhagen. Denmark

Keywords: nutrition policy, diet and health information system, food quality, labelling, food safety

Diet and health in Europe

After the devastations of the Second World War in Europe, there was a steady increase in the availability of food in the Region, and, except in Eastern Europe, a steady increase also in the variety of foods available.

In the same period of time, there has been a marked change in the dietary pattern in all countries, and these changes are remarkably similar in all of the countries in the Region. As demonstrated by FAO's food balance sheets, which are the only internationally comparable set of data on food trends in Europe, since 1961 there has been a trend towards a decreased consumption of staple foods, which in Europe is cereals (Fig. 1), and in Northern Europe, potatoes (Fig. 2). At the same time, there has been a marked increase in the consumption of meat (Fig. 3) and milk (Fig. 4). The trend in fruits and vegetables mostly show an increasing tendency (Fig. 5), while alcohol consumption has increased dramatically in the period.

This overall picture of course conceals large regional differences. And although the change is rather drastic as demonstrated here, people in the countries concerned do not necessarily realize how much their diet has changed. In Denmark where the author lives, people will insist that they eat for example meatballs and potatoes just as their ancestors always did. While that is actually true, there is a difference in that while the ancestors had three potatoes and some meatballs on the plate, today's Dane has three meatballs and one potato.

The changing dietary pattern has had a rather dramatic effect at the level of nutrients. The percentage of fat in the diet has increased, and although

[a] Paper presented at the International Symposium on Food, Nutrition and Economy Development in China, 1990.

our data are not too good, there is reason to believe, that the proportion of fat from saturated fatty acids has increased as well. Especially in Southern Europe this has had a marked effect. While in earlier days olive oil with its large proportion of monounsaturated fatty acids was the main source of fat

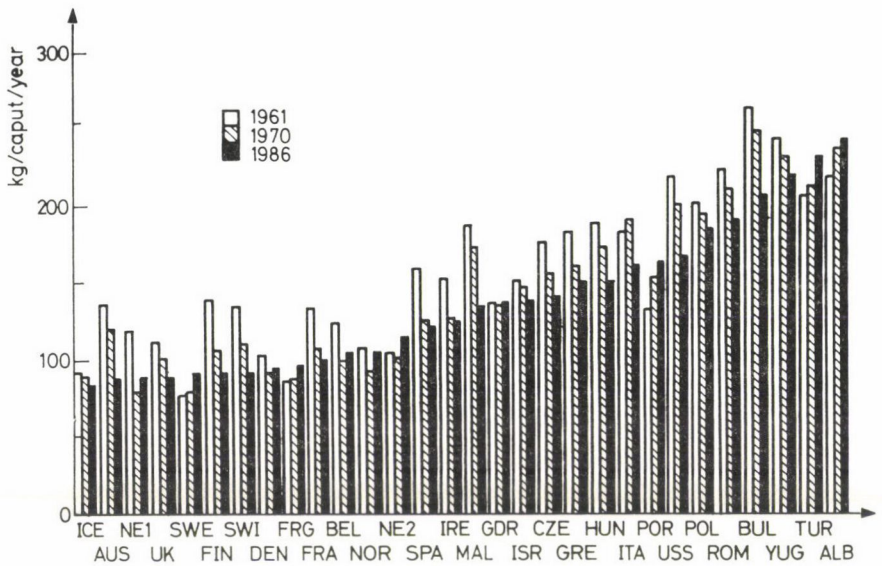

Fig. 1. Consumption of cereals and pulses in Europe

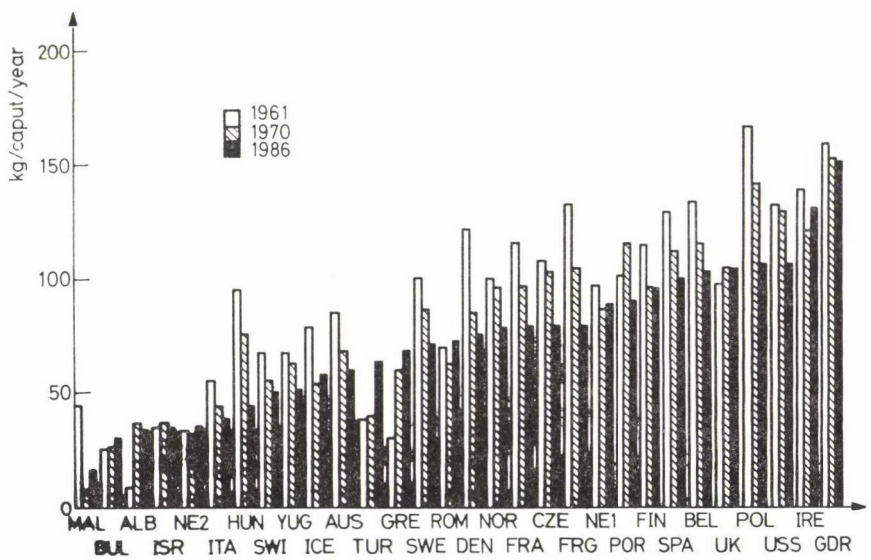

Fig. 2. Consumption of potatoes and tubers in Europe

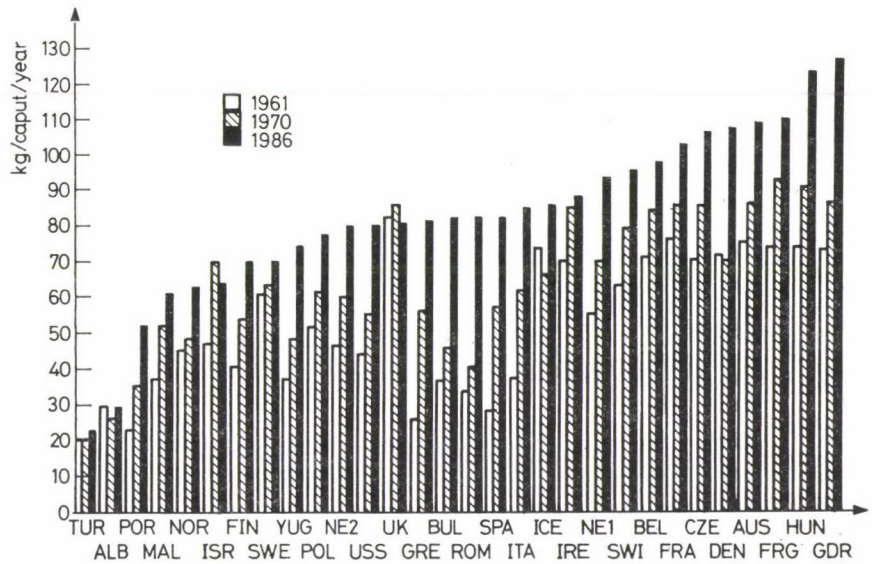

Fig. 3. Consumption of meat, poultry and eggs in Europe

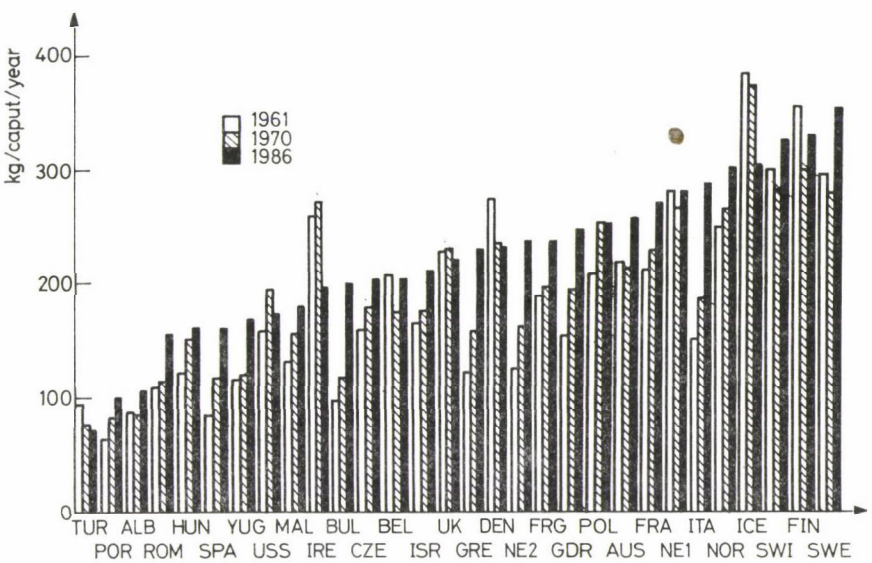

Fig. 4. Milk consumption, excluding butter

in the diet, today the proportion of saturated fatty acids is considerable. In Eastern Europe, the meat intake is rather high, in some of the countries even very high, and it has been so for a long period. The meat however is rather fatty. Unlike in the western parts of Europe, where animal breeding has

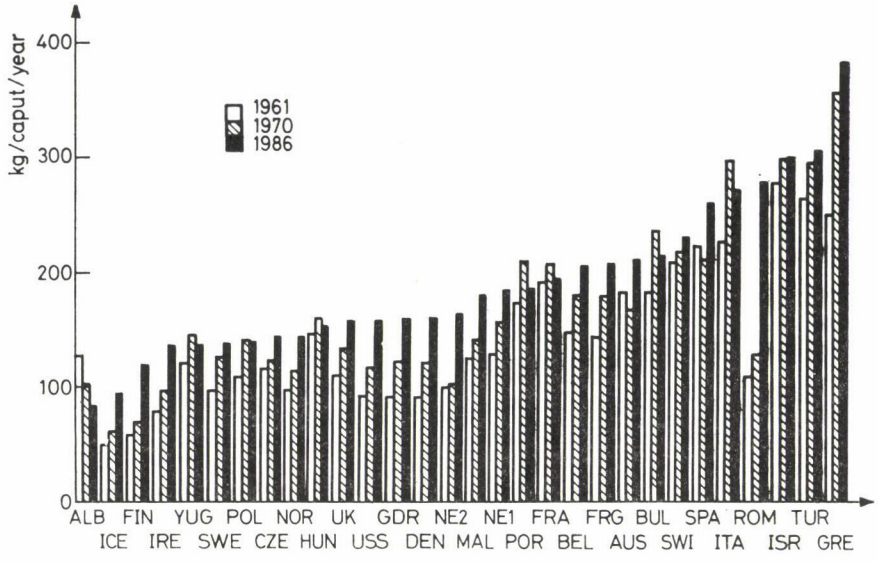

Fig. 5. Vegetables and fruits consumption in Europe

strived towards leaner varieties of animals, especially of pork, a similar development has not taken place in the East. Even in Romania, with its many problems, where meat was exported in the last years to pay for the foreign debt, animal fat in the form of lard was easily available, and eaten with bread it was said even to have caused obesity in the population (WHO, 1990).

There is also large variability between the European countries in vegetable and fruit consumption. Southern Europe has traditionally had a high consumption because of easy availability, while in the North people traditionally consumed relatively small quantities of mainly coarse vegetables such as cabbage and turnips, and fruits such as apples and cherries. The North has seen a shift from coarse to fine vegetables, and an overall increase in vegetable consumption, whereas in the South the consumption may proportionately be somewhat lower than it was traditionally. These changes of course will have consequences for intakes of vitamins and minerals, and of whatever factors in these food groups that may possibly have a cancer preventive effect.

Turning thus to the health situation in Europe, again we are faced with a changing picture. The most marked and consistent change in the period after the Second World War is the increase in premature mortality from cardiovascular diseases, especially ischaemic heart disease. The increase in the postwar period has been said to take on almost epidemic proportions, and the diseases struck as is well known mainly men in their middle ages. In northern Europe the increase started first, so by the mid-1980's there began to be signs of a levelling off of cardiovascular mortality in many Northern European

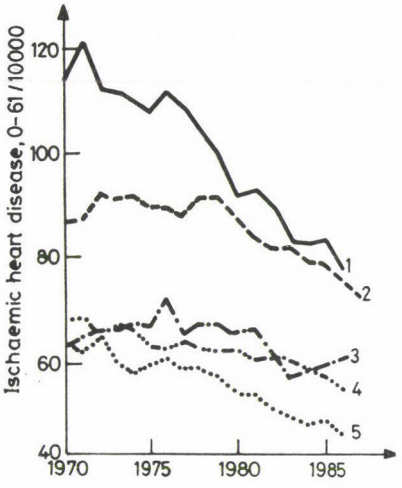

Fig. 6. Cardiovascular mortality in some Northern European countries.
1: Finland; 2: U.K; 3: Denmark; 4: Norway; 5: Netherlands

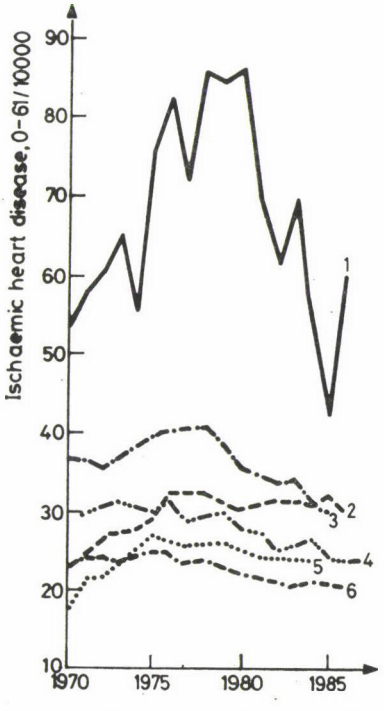

Fig. 7. Cardiovascular mortality in some Southern European countries.
1: Malta; 2: Greece; 3: Italy; 4: Portugal; 5: Spain; 6: France

countries. This later developed into a clearly decreasing trend in some of the countries which were previously very high, such as the Nordic ones, and England, Scotland and Wales (Fig. 6). In Southern Europe, where premature mortality rates from these diseases had been rather low compared with the

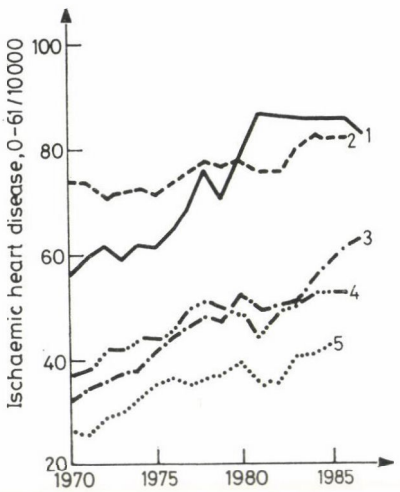

Fig. 8. Cardiovascular mortality in some Eastern European countries.
1: Hungary; 2: Czechoslovakia; 3: Poland; 4: Bulgaria; 5: Yugoslavia

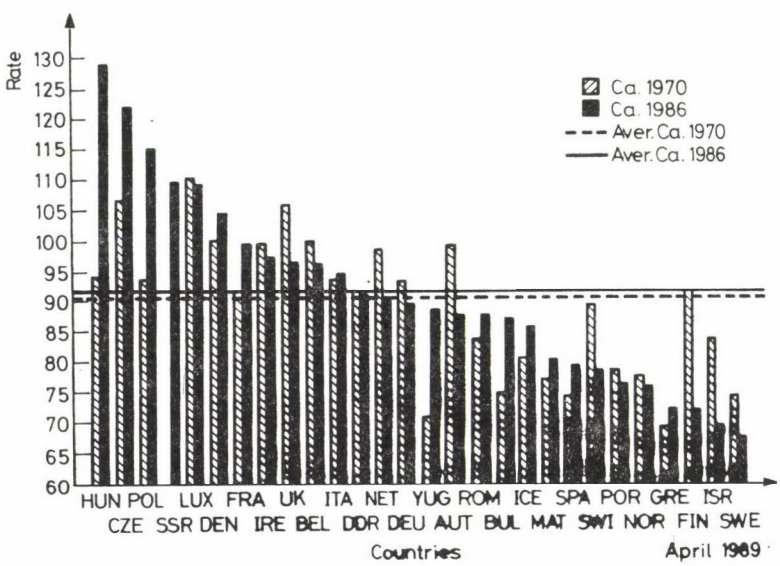

Fig. 9. Standardized mortality rates, all malignant neoplasms in Europe, age group 0–64. Both sexes, Ca. 1970, 1986. Source: HFA database, EST/EURO; note: data for SSR ca. 1970 not available

North, there was a sharp increase in the 1960's and early 1970's, but again a levelling off of the increase by the early 1980's and even a decreasing trend throughout the 1980's (Fig. 7). Eastern Europe again had an unfortunate development, with rather sharp increases in the course of the 1970's and 1980's, and only those at the top of the dubious ranking list, such as Hungary, now sees a tendency towards levelling off of the high premature mortality from cardiovascular diseases (Fig. 8).

Cancer mortality also varies throughout the Region (Fig. 9), but there is a general tendency towards a decrease in stomach cancer mortality, and an increase in color cancer mortality, with a variable picture for cancer of the breast, though an increasing tendency is notable in Southern and Eastern Europe. Sadly, cancer of the lung is clearly increasing in most countries, with a few exceptions such as Finland and the United Kingdom (MASIRONI & ROTHWELL, 1988).

This paper does not discuss the relationship between diet and health, and the above data have been selected on the basis of our present indications of relationships as expressed for example in two recent reports from the USA (SURGEON GENERAL, 1988, NAS, 1989) as well as Europe (JAMES, 1988). No one will however deny that the relationship between diet and health is complicated by the concurrent effects of several factors, such as physical activity and smoking. Even so, we have interesting indications from countries in the

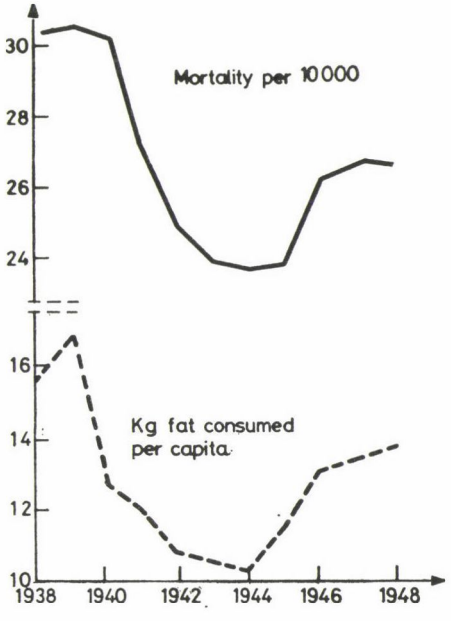

Fig. 10. Mortality from cardiovascular diseases and fat consumption in Norway 1938–1948 (Source: Ström A and Adelsten Jensen R 1949)

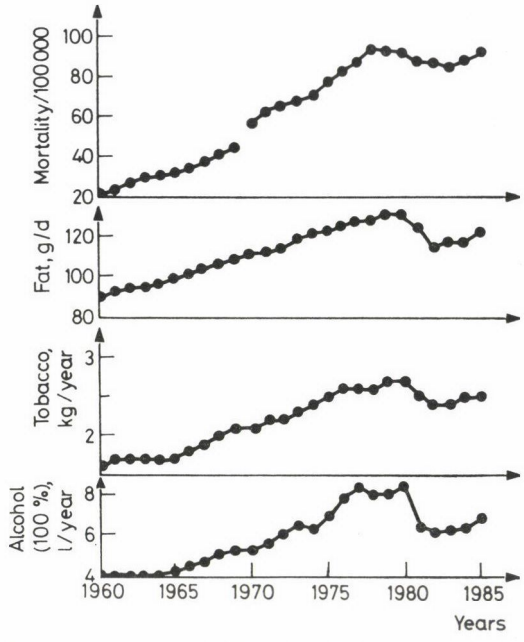

Fig. 11. Ischaemic heart disease mortality, total tobacco and alcohol consumption in Poland 1960–1985 (Source: Szostak WB and Cybulska B Am J Clin Nutr 1987; 45)

Region that there is a rather immediate effect of frugal lifestyles upon cardiovascular mortality. Figure 10 shows the situation in Norway immediately following the Second World War. The parallel development of the dietary fat intake and mortality from heart diseases is striking. A similar picture from Poland is shown in Fig. 11, where forced frugality had a remarkable effect on ischaemic heart disease mortality in the period 1976 to 1981 when the situation was rather bad, followed by a "rebound" effect in the course of the 1980's as the situation improved.

In summary, although there is a lot of similarity between the general trends in the European Region with regard to diet and health, there are distinctive differences between geopolitical regions, especially in the North, the South and the East. There are naturally also differences within these regions, although they are more subtle.

Nutrition policies in Europe

A nutrition policy is here defined as a set of coordinated actions, based on a governmental mandate, to ensure the nutritional quality, and safety of the food supply to the population. The intention of these actions may also

explicitly be to promote health and improve dietary habits. (WHO, 1988). Nutrition policies were actually implemented in many European countries after the Second World War, when Ministries of Agriculture sought, and got, the advise of nutrition experts as to what they should cultivate in order to optimally supply their populations. Such policies were in operation in, for example, the United Kingdom, Greece and Norway (HELSING, 1990). As the food supply situation improved, however, such advice was no longer sought, and nutrition policies for a long time were thought of mainly as relevant to developing countries (BERG & MUSCAT, 1973).

In the early 1970's FAO put considerable intellectual resources into the analysis of nutrition policies, and developed complex models for the intersectoral interactions that decided food supplies (GANZIN et al., 1973, LUNVEN & BOKOBO, 1974).

At the World Food Conference in 1974, however, a resolution was adopted stressing the need for all countries to adopt food and nutrition policies regardless of stage of development. At the Conference, Norway announced its intention to soon adopt such a policy. As the work with the Norwegian food and nutrition policy was by then almost finished, it was adopted by Parliament in 1975 (NORWAY, 1975).

For a long time no other country followed suit, although the United Kingdom also had announced its intention to formulate a nutrition policy at that time. In 1984 however the Governments of the Netherlands and Denmark, both members of the EEC, each adopted a national nutrition policy (NETHERLANDS, 1984; DENMARK, 1984). In 1988 Malta's cabinet adopted a Food and Health policy for Malta (MALTA, 1988), and in 1989 the Icelandic Allthing passed an Iceland nutrition policy (ICELAND, 1989). At the moment Finland is working on the formulation of a Finnish food and nutrition policy, as announced in the Finnish Health for All Policy which was adopted in 1985 (FINLAND, 1987).

The situation in the Region today, then, is that five out of the thirty two Member States have a food and nutrition policy. This is possibly more than in most other WHO Regions. Also, the policies are still in the process of being implemented, albeit in a slower tempo than most of their proponents might prefer.

A comprehensive nutrition policy

In the WHO Regional Office for Europe, the Nutrition Programme has since 1984 worked primarily with the conceptual development of comprehensive nutrition policies. A general organizational framework in the form of a simple model has been formulated, reflecting all the areas of action contained within a national food and nutrition policy. The reason for drawing

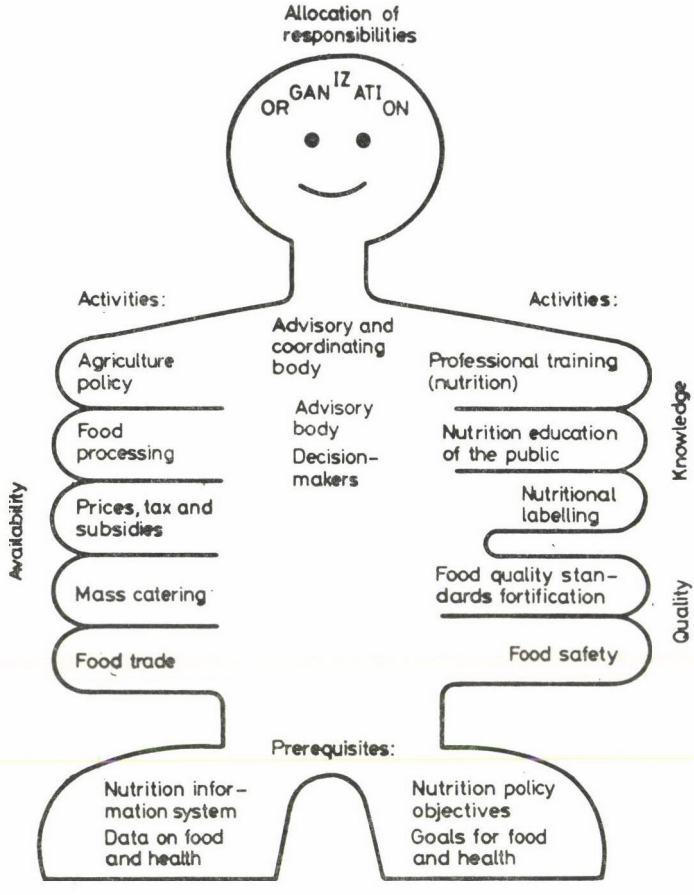

Fig. 12. Holistic homonculus of nutrition policy

up this model was in the first place to help organize discussion and action around the complex subject of nutrition policy. Somewhat lightheartedly, but with a serious intent, this has been drawn up in the form of a humanoid figure, "the holistic homonculus of nutrition policy" (Fig. 12). This concept has been presented and discussed on numerous occasions in the European Region (HELSING, 1988, 1989).

Objectives and an information system

According to the framework, a nutrition policy seems to require, before it can be implemented, clear and explicit objectives indicating what one expect to obtain through policy implementation, in the area of health and in the area of nutrients, and how this should be reflected in the development

of the food supply. Such objectives must obviously be based on the situation in each country as it is, so policy-makers must have an information system at their disposal for monitoring of the effects of the policy. An information system does not have to be complicated, much of the data is already collected by others, for example in household budget surveys or agriculture statistics (BECKER & HELSING, 1991). As they may be regarded as foundation for a comprehensive food and nutrition policy, the setting of objectives and use of an information system are drawn on the figurine as its right and left foot, respectively.

Areas of policy implementation

The areas of implementation activity are pictured as the "hands" of the holistic homonculus. Three categories of implementation activities, or policy measures, can be distinguished: those that deal with the availability of foods, those that concern knowledge about foods, and those that consider the quality of foods.

Agriculture policies may to a large degree determine the composition of a national food supply. In the last thirty years European agriculture policies have mainly been shaped according to criteria such as their economic implications for farmers, employment and military security. Health has usually not been seen as a relevant criterium to bring into the discussions of agriculture planning.

Food processing and manufacturing has gone through a rapid technical development in the last thirty years, and has today unprecedented potential for the processing of healthful food. As the health consciousness of consumers and hence the food market has increased, the food manufacturing sector may have strong incentives for collaboration with the health sector. There is thus considerable potential for positive collaboration with this sector in nutrition policy implementation (NAS, 1989).

Mass catering is a growing sector in most European countries. With changing family structures and family economies, people take a larger share of their meals outside the home. Both private and public mass catering establishments can contribute to improved nutrition, and there is large potential for small but important changes especially in ordering, preparing and serving foods in such establishments. Often the customer may not even notice that the food has been nutritionally improved (WHO, 1987).

Food prices are a result of a variety of factors, and they are often set partially by Government interventions such as subsidies at production level. Practically in all countries there is some form of Government interference with food prices at one level or the other, either as taxes or as subsidies. In countries with a Governmental mandate for a food and nutrition policy, those

responsible for price setting should take health into consideration as a decisive factor when food prices are set (WHO, in press).

Food trade, import and export as well as internal transportation systems are all decisive for who will get what foods. For example, the lack of fruits and vegetables in the former communist countries in eastern Europe are apparently partially the result of a poor internal distribution system, as all of these countries have a large potential for fruit and vegetable production. In nutrition policy implementation this is a very important area.

All of the above areas together decide what foods shall be available to the consumer. The food consumers, regardless of their knowledge about food and nutrition, are rather dependent on the selection of foods that are made available to them. What is available is usually what will be eaten. In countries with a nutrition policy, all of the above action areas should logically be related to the objectives of that nutrition policy.

Training of professionals in food and nutrition science is a subject of great concern in Europe. The nutrition science is not well developed in many European countries, and there is a great shortage of professionals who can provide scientifically well-founded advice to all those nutrition policy actors described above. The collection and analysis of nutritional data, too, depend on a critical and well developed corps of trained nutrition scientists. Many European countries have far too few such scientists available. Consider that in the USA, every year several hundred persons are receiving an academic degree in nutrition, while in the USSR the figure is zero !

Nutrition information campaigns for the public in a nutrition policy context are mainly taken to mean campaigns that are run by Government agencies. In most countries today, the food industry completely dominates the nutrition information scene, and one might well think of joint ventures between nutrition policy-makers and the food industry in a food and nutrition policy context, when industry and policy-makers have common objectives, which is actually quite often. In countries with a long experience with nutrition policy implementation this has from time to time been a useful type of alliance for specific campaigns.

Nutrition labelling of foods will probably be increasingly important as foods are being processed and packaged to a larger extent than earlier. Labelling serves both to identify the food and to convey messages about its nutrient composition. Nutrition labelling presupposes an educated consumer, but may also in itself have an educational effect. Eventually nutrition labelling will probably be mandatory, but so far most European countries have mandatory labelling only of foods for special dietary uses or foods when nutritional claims have been made.

The three implementation measures mentioned above all comprise activities that concern knowledge about diet and health, about food and

nutrition. In a nutrition policy context these measures should logically all be related to or based on the objectives of the policy.

Food quality standards comprise standards set to regulate the ingredient composition of foods, as well as the fortification, enrichment or restoration of nutrients in foods. When related to nutrition policy objectives such standards could be one important contributor to a better nutrition in the population.

Food safety in itself is an important contributor to microbial and biochemical safety of foods, and an important aspect of a food and nutrition policy. Regrettably, since food safety regulations were worked out long before other aspects of the relationship between diet and health were fully appreciated, few countries if any have managed to combine their food safety policy with a nutrition policy. Ideally the two would belong together, but there are in most countries administrative difficulties to be solved before this could become a reality.

The two latter implementation measures may not in the past have been used to their capacity for nutrition policy implementation. In most people's minds food safety equals nutrition policy, and although nutritionists do not contest the importance of this subject, they tend to regard for example additives and pesticides in food as much less of a threat to general health than, say, saturated fatty acids.

European nutrition policy-making at present

As indicated by the "head" of the homonculus, organization of the nutrition policy, or allocation of responsibility for coordinating and carrying out a nutrition policy is a central feature of nutrition policy-making. In Europe there is at present a variety of solutions to the organization of nutrition policy. In the following we will try to demonstrate the various models by describing some experiences in policy implementation in Europe.

Norway

Motivation for the policy. When the "Food supply and nutrition policy" was adopted by the Norwegian Storting, or Parliament, in 1975, it represented a joint effort between the Ministry of Agriculture and the Ministry of Social Affairs (NORWAY, 1976). The document itself was written by a multisectoral group consisting of a variety of agriculture specialists and nutrition experts. The involvement of the agriculture sector in nutrition policy was important, and probably a result of the need for this sector to use the nutrition policy as one of many arguments to justify the large income transfer into agriculture that was foreseen in order to keep the sector economically and socially viable. Formulated at a time when world food resources were assumed to be critically

low, the policy also reflected a real concern about food security in a small country which is not food self-sufficient and thus dependent on food importation.

Organization of the policy. A central institution in the period leading up to the policy adoption had been the Norwegian Nutrition Council, established first in the 1930's, and re-established in 1946 at the initiative of among others FAO. The Council throughout the course of its function has had broad representation not only from nutrition expertise but also from the food industry, agriculture and farmers organizations, consumers and trade. The Council always has had a small secretariat, which was slowly strengthened in the course of policy implementation, and which has in many ways been the most important focal point for carrying out the Norwegian Food Supply and Nutrition Policy. Its annual reports constitute a continuous monitoring of policy implementation efforts and results.

As the policy was, from the outset, seen as a multisectoral effort, a specific body was established to take care of interministerial collaboration in policy implementation, called the Interdepartmental Coordinating Council (ICC). Unlike the Nutrition Council, the ICC did not have a full secretariat, but was placed administratively under the Ministry of Social Affairs. The ICC met seldom, took no decisions, and is now largely regarded as defunct.

In summary, the Nutrition Council, representing a broad spectrum of interests, became the executive body for Norwegian nutrition policy implementation, rather than being limited to an advisory function as the title

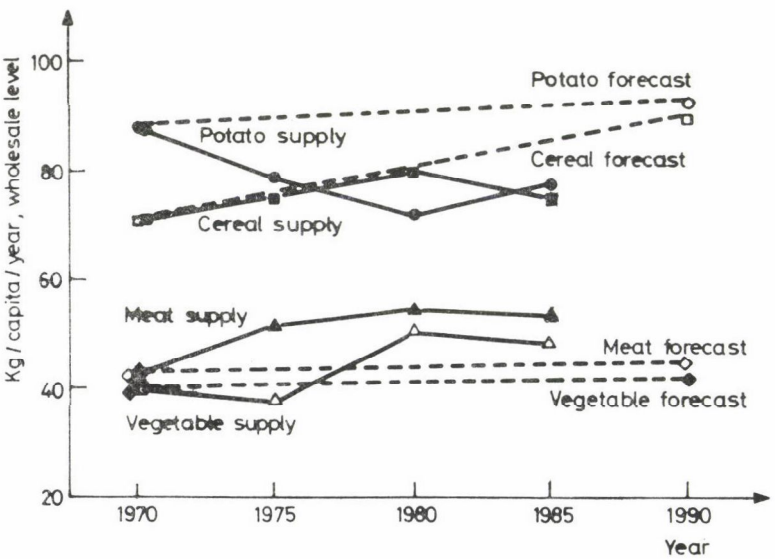

Fig. 13. Food in Norway 1970–90. Forecasts and developments (Report of the Norw. Nutr. Council, 1985)

might imply (MILIO, 1988). The ICC was never given the resources necessary to function and consequently did not do so either.

The policy has had some effect on the food supply of the country, in that Norway is one of the few European countries where the fat content of the population's average diet has decreased in the course of the last ten years. The long-term food forecasts drawn up in 1975 (Fig. 13) have been useful in advising producers and manufacturers in which direction the food supply should develop, and an analysis of the situation today shows that the changes in the food supply stem from a number of small changes in many areas.

The Netherlands

Motivation for the policy. Public concern with the safety of food and the relationship between diet and health led to political interest in the issue. A new Directorate was established in the Ministry of Welfare, Public Health and Cultural Affairs, responsible for nutrition. A report to the ministry in 1983, entitled "Food and nutrition policy in the Netherlands" (NETHERLANDS, 1984) described these two aspects of food and nutrition in great detail. Emphasis was placed on allaying public fears about contamination of the food supply, which is not a real problem, and drawing attention to the composition of the diet, which is a real problem. The report and its proposals for action were adopted by Parliament in 1984, and is the basis for the Dutch food and nutrition policy. The nutrition and health part of the policy places main emphasis on description of the food and nutrition situation, on public education, and on research.

Organization of the policy. The practical implementation of the policy is entrusted to the new Directorate mentioned above. Its various elements are however implemented by a variety of institutions. The food safety elements of the policy are the responsibility of the Ministry of Agriculture, while the health elements are naturally the main focus of the Ministry of Public Health. The main scientific advisory body to both ministries is the Nutrition Council, which is composed purely by scientists and does not represent any vested interests. Its recommendations are of consequence for the implementation process. It is responsible among others for nutritional guidelines for the population (NETHERLANDS, 1986). The Bureau for Nutrition Education which already before the adoption of the policy was a rather large establishment, is responsible for the public education element of the policy. The research element as well as the description of the nutrition situation is taken care of by universities, by the State Institute for Public Health, and by the Central Institute for Food Research.

The Parliament has requested reporting at regular intervals of about three years on the implementation of the Policy, and the first follow-up report

was submitted in 1987 (NETHERLANDS, 1987), the second was published in 1991. When the last review was discussed in Parliament, the progress made was considered to be satisfactory, and it was stressed that particularly the behavioural sciences should play a greater role in research (VAN DER HEIDE, 1989). The implementation of the nutrition policy has also had a stimulating effect on the nutrition education work (PAS, 1988).

Malta

Motivation for the policy. Although a Mediterranean country, due to its history with a high degree of northern European influence the Maltese diet has many traits in common with that of the north (VUKSAN et al., 1982). The pattern of mortality from non-communicable diseases, too, is more like that of the northern European countries (BELLIZZI, 1989). Awareness of this among Maltese health policy-makers led to the establishment, in 1985, of a Nutrition Unit in the Department of Health. Its dynamic leader organized two national Conferences on Nutrition in Malta in rapid succession, in 1986 and 1988. This created considerable political awareness about the situation, so that late in 1988 the Cabinet was ready to adopt a multisectoral nutrition policy.

Organization of the policy. A characteristic feature of the Maltese nutrition policy is its intersectorality. A conscious effort has been made to involve education, agriculture, industry and trade in the initiative taken by the Department of Health. In both of the Conferences mentioned above, these sectors have been actively involved. A national multisectoral advisory cum administrative committee was set up in 1986, with a mandate to formulate and implement the nutrition policy. The committee, the National Committee on Food and Nutrition, thus combines an advisory and an executive function. This combination may be practical and relevant in a situation when there are few resource persons to draw on. After a few years of experience with policy implementation, the head of the Nutrition Unit stresses the importance of clearly formulated policy aims and objectives, so as to help policy-makers gauge the success of the policy. Further, responsibilities for carrying out recommended measures must be spelled out and assigned to the appropriate key persons, committees or organizations (BELLIZZI, 1989).

Denmark

Motivation for the policy. A food producing and exporting country, Denmark has a typical abundant, high-fat, rapidly changing dietary pattern. In contrast to most other European countries, Denmark has never established a nutrition advisory council, nor was there in the early 1980's any academic institution solely devoted to nutrition research. Denmark however took part

in the inter-Nordic nutrition work within the framework of a Nordic Ministerial Council's food and nutrition programme. In 1979 the National Food Agency decided to strengthen its Nutrition Unit, which expanded rapidly in the early 1980's. Although the nutrition work in Denmark was at that time very fragmented, there was a strong interest in the subject in the population, and several proposals for comprehensive nutrition policies were formulated by different bodies.

Organization of the nutrition policy. In October 1983 the Social Democratic Party, the largest opposition party in the Danish Parliament, formulated a proposal for a Danish nutrition policy, which was adopted in May 1984.

Unlike the policy documents mentioned above, the Danish document is a somewhat amputated policy. It does formulate five proposals for activities, assigns them to different bodies, but the policy does not contain any proposal for follow-up. Nor does the policy document state specific goals for the nutrition policy or assign any responsibility for overall scientific advice or coordination of the various activities. The activities proposed are in the areas of nutrition research, mass catering, food safety, nutrition information of the public and mapping of the dietary patterns of the Danish population (DENMARK, 1984).

The five areas for action were actually implemented: a Centre for mass Catering was built up in 1986, and in 1989 became a WHO Collaborating Centre for Mass Catering. A Research Institute for Human Nutrition was established in 1987, and work in the area of food safety progressed as it had before the policy adoption. Nutrition information of the public did not receive particular attention although a programme for its implementation was worked out but never funded. A large-scale dietary survey was carried out in 1985 (HARALDSDÓTTIR et al., 1986).

Nutrition continued to be politically important, though, and when in 1988 an action plan for a Danish "Preventation Policy" was formulated and later adopted by Parliament, its section on nutrition and food further elaborates the originally very general goals of the Danish Nutrition Policy. Still to this date, however, there is no overall coordinating body for the Danish Nutrition policy, nor is there an advisory nutrition council. The Danish nutrient pattern has, unlike for example the Norwegian, not changed over the last five years.

Iceland

Motivation for the policy. The Icelandic Health for All policy adopted in 1987 (ICELAND, 1987) states that nutrition is an important part of the policy, so in 1989 the Icelandic Parliament, the Allthing, adopted a nutrition policy (ICELAND, 1989). The current Minister of Health, who has the backing of the Agrarian Party, strongly endorsed and promoted the Policy. Thus in Iceland,

as formerly in Norway, the agriculture sector initiated the nutrition policy, perhaps seizing the initiative so as to be able to steer events.

Organization of the policy. The Policy document states both general and specific objectives in clear terms, and sets out areas for action, which comprise in addition to education also price policies and trade, as well as importation which is important in the case of Iceland. A dietary survey is to be carried out as a basis for further implementation strategies. Responsibility for implementation of the policy lies with the Ministry of Health, which has a five-year reporting obligation to the Allthing. The further organization of the implementation work is currently delegated to the Icelandic Nutrition Council, which was first established in the early 1930's, disappeared in the early 1940's and was re-established in 1978. It is somewhat early to describe the final organization structure, as well as to expect any results of the Policy, though work is under way to carry out the dietary survey, public education has started and courses for fast food outlet owners and health workers in the principles of modern nutrition area being prepared for execution in 1990.

Conclusion

Nutrition policy-making in Europe is at an early stage of implementation. Most of the existing policies were adopted in the course of the 1980's. We are only just beginning to learn the art of this policy-making, and to study it scientifically (MILIO, 1988b). The political interest in this type of policy is however clearly increasing, and the First European Conference on Food and Nutrition Policies, organized by the World Health Organization's Regional Office for Europe in Budapest in October 1990, generated a lot of attention.

In the long run, food producers and manufacturers, faced with an increasingly health conscious market, needs the alliance with the health profession. The health profession needs a nutrition policy so that the translation of their scientific knowledge into political action can take place in a reasonably systematic way.

Patience is probably still called for. The Maltese head of the Nutrition Unit which is charged with nutrition policy implementation in Malta, in a recent article concluded: "Nutrition policy makers should keep in mind that other sectors have their own objectives, which do not necessarily coincide with those of the nutrition policy. It may therefore take time for the alignment of other policies to the nutrition policy. The best advice is to retain patience but to persevere. Accept the fact that the nutrition policy can succeed in some areas at a faster rate than in others, but it will never happen overnight." (BELLIZZI, 1989).

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AGRICULTURAL POLICY IN EUROPE: HOW DO THEY INTERFACE WITH FOOD AND NUTRITION POLICIES[a]

J. S. MARSH

Department of Agricultural Economics and Management, University of Reading, 4 Early Gate, Reading RG6 2AR. United Kingdom

Keywords: nutrition policy, food economy, environment, information

1. The case for a nutrition policy

From its earliest days the Food and Agriculture Organisation of the United Nations has been concerned with the ability of people to acquire an adequate supply of food. Initially the problem for most of the world seemed to be how to produce a sufficient quantity of food to meet the requirements of growing populations. For many of the poorer countries of the world that remains a key issue but elsewhere other matters have become of growing significance; for example, the relationship between economic growth and the ability to buy food, the distribution of food within as well as between countries and the implications of the increased food production for the environment. Among these issues is the impact on health of human diets.

Within most of Europe there has been no general problem of food shortage since the early 1950's. However, there has been a growth in cardiovascular diseases, cancer and diabetes melitus all of which we have now come to understand are associated with diet. They are now called "the diseases of affluence", indicating their relationship to choices people make when income are high and food abundant. There is thus a need to explore ways in which consumers may be helped to chose a healthier diet. Government actions in this field form part of what is referred to as a "nutrition policy".

Nutrition policy must include availability, stability and accessibility. In this context availability requires the production of sufficient food to ensure that all can enjoy a satisfactory diet. Stability implies that food must be available in sufficient quantities when it is needed, throughout the year despite the seasonal nature of much production. Accessibility implies that everyone has sufficient resources to buy or produce the food needed. These

[a] Paper presented at the WHO Conference, Budapest, 2–5 October 1990.

general goals have been supplemented by more specific guidelines about the nature of diets which may enhance human health. In particular in much of Europe, where the quantity of food consumed has been such that obesity rather than hunger has been a problem, guidelines have focussed on the need to reduce the proportion of fat, especially saturated fats, sugar, salt, and alcohol in many people's diets. These items would be replaced at least in part, by the consumption of more fruit and vegetables.

Such guidelines interface with many other policies, including education health and social security. Their effects have a direct implication for the food and agricultural industry and for agricultural and food policies. This paper explores these links, discusses how the policies may develop and considers how nutritional issues might play a role in their future development.

This paper attempts to set out some of the implications of adopting a policy which gives greater priority to nutritional considerations for the agricultural industry of Europe. It discusses:

- The objectives of food and agricultural policy
- The successes and failures of recent agricultural policies
- New influences on agricultural policy
- The implications of nutritional guidelines for agriculture
- Where should policy go now?

1.1. The objectives of food and agricultural policies in Europe

Governments have intervened in agriculture and the food sector for a variety of reasons and over many centuries. Europe is made up of many nations and their agriculture and food problems are not the same. In much of the west of Europe, food shortages no longer exist, food surplus has become a problem. In the south of Europe, where incomes have been lower and the diet much more based on vegetable foods, diet in nutritional terms is more satisfactory. Growing affluence is now leading to a pattern of consumption more typical of the affluent north. In Eastern Europe, shortages of some foods have been apparent in queues at food shops and in the requirement to import food. Deficiencies in food quality and the post harvest wastage of food represent less obvious but serious problems. It is possible, however, to identify some common issues which currently influence policy in most European countries, although often in different ways. These include:

1.1.1. A concern for economic efficiency. The wealth of society depends upon the use it makes of its resources. Changes in demand and new technologies require the movement of some resources from their current to different uses. By facilitating this movement governments can help to raise the level of national income and, provided income distributions are appropriate, increase the living standards of the population. In Western Europe in the past two

decades economic efficiency has implied a shift in the volume and mix of resources used on farms. New, more productive farming methods have enabled the output of the industry to grow despite a major reduction in the number of people employed, a reduction in the area of land farmed and falling real prices for farm productions. Part of the increase has been due to the substitution of capital for labour but overall the resources released have been able to make a contribution to growth in other sectors of the economy.

Gains in economic efficiency have only been attainable with changes which have had social and political consequences unwelcome to many Europeans. Changes in the character of rural society, the depopulation of some regions, the shift in the balance of occupations to non-farming activities and the pressure on the small family farmer to become part-time or to leave agriculture have been resisted.

Changes in agriculture have impacts which are not reflected in the market price of food but which affect the value of agricultural activity to society as a whole. Such changes include increased risks of pollution resulting from the mis-use of pesticides, herbicides and fertilisers and from intensive livestock production. The power of modern farming to change the physical environment has led to the loss of some wildlife habitats and unwelcome changes in the traditional appearance of the landscape. There is also considerable concern about the sustainability of a system which relies on the use of fossil fuels and may, if modern methods are not used with care, lead to irreversible damage to the soil. Such developments mean that the gains in efficiency reflected in the market price of food may overstate the benefits society receives from these changes in farming. Policy may then seek to modify the way in which resources are employed to take account of these "externalities."

The impact on the health of the population can be regarded as another area in which the "externalities" of the agriculture and food industry are of importance. Increased costs of medical care, early death and the distress involved in disease all reduce the real wealth of society. In evaluating the economic benefit of agricultural change these costs have to be taken into account. If by providing incentives the industry can persuade consumers to adopt a more healthy diet, then economic as well as social benefits may result. Of course in such a calculation allowance has to be made for the costs which society will ultimately have to face as the population ages and eventually dies from other causes.

1.1.2. Economic management. In Europe agriculture and food remain major sectors of the economy. Although spending on food tends to decline as a proportion of total consumer expenditure the impact of changes in employment, prices and trade in these industries remains important. Agricultural policy has been used to provide more stable prices, to replace imports or increase exports and to continue in employment some of those who in a freer

market might have been forced to seek other jobs. In Eastern Europe priority has been given to holding down the price of food. One result has been that a large share of public expenditure has been devoted to food subsidies. Under the Common Agricultural Policy (CAP) of the European Communities (EC) import levies and export subsidies have had the effect of keeping the internal price of food relatively stable. Because prices have been fixed at levels at which, given access to productivity increasing investment, farmers have been encouraged to expand, one result has been the creation of surpluses. These have had high budgetary costs and tended to depress world markets.

1.1.3. Distributional goals. Governments are impelled by political pressures as well as by social need to interest themselves in the distribution of income. The unfettered operation of market forces may leave some groups with income levels which are regarded as unacceptably low. Income may be redistributed by taxation and social security policies but in Europe much policy relating to food and agriculture has been concerned with distribution. In many of the Centrally Planned Economies (CPEs) price levels have been held down as a means of ensuring that the real income of poorer consumers is supported. The economic consequence of these policies is to transfer income from other sectors of the economy to the beneficiaries. It is arguable that such devices are an inefficient instrument of support as rich farmers and rich consumers receive relatively large shares of the benefits, whilst the losses of competitiveness in other sectors of the economy reduces the income earning potential of poorer people. (See for example Bureau of Agricultural Economics, Intersectoral effects of the CAP: Growth, Trade and Unemployment, occasional paper no. 95, Canberra 1985.)

1.1.4. Food supply goals. The provision of a secure supply of food is a basic requirement for all societies. The concept of food security is usually in terms of the availability of continuing supplies of familiar foodstuffs rather than in strictly nutritional terms. Hence a failure, for example, in the supply of sugar, which might be welcomed on nutritional grounds, is regarded as a political disaster. If a strictly nutritional view were taken of food security, then the volume of food required would be much reduced. The substitution of vegetables for some animal based foods in the diet could release very large quantities of nutrients which currently enter into animal feedingstuffs.

Food supplies may be secured directly by producing the food at home or indirectly by producing other products and exchanging them for food in the world market. In practice, despite economic evidence which suggest that trade might be the least cost way to ensure food security, most governments have preferred to pursue policies which ensured a high degree of self-sufficiency. One implication of this has been the distortion of international trade. Prices in world markets are often below those offered to domestic farmers, barriers to imports and subsidies are required to make exports possible.

Apart from making sure that sufficient food is available governments have also been responsible for ensuring that food is safe to eat. Food safety includes freedom from micro-biological hazards, from pesticide residues and from contaminants such as heavy metals. Consumer confidence in the food supply requires assurance that, despite changes in the methods of agricultural production, food processing and distribution, the food which is eaten is not damaging to their long run health.

The accidental release of radiation at Chernobyl has increased awareness of new complications for food security and food safety in a nuclear age. It is still impossible to assess the full consequences for food production of this event but already it demonstrates that relatively modest releases of radiation may render unproductive, so far as food is concerned, considerable areas of land for several years. It has also drawn attention to the need to have accurate standards relating to the level of radiation in food which is safe so far as humans are concerned and an effective means of monitoring these standards. Whilst the primary aim of all policy must be to avoid such events, food policy makers need to have a strategy about how best to cope with them should they occur.

Governments have also concerned themselves with food quality. Improvements in quality in a competitive industry are one means of securing a larger share of a market which, in aggregate terms, is static. Quality improvements affect all sections of the agricultural and food sector. At a farm level the development of leaner breeds of livestock provides one example. Within the food industry quality demands attention to the storage, grading and packaging of food products. In the retail shop quality and competitive strength include such matters as the selection of food, the way in which it is presented and the avoidance of mechanical damage. Quality for some consumers includes consideration of the way in which the food is produced as well as the content of the food itself. One manifestation of this is the search for "organic" food produced without the use of chemicals. The market in such organic foods is growing and although currently accounting for only 1.1% of grocery sales in the EC. (Corbett D. in: Eurofood, 1990 April.) It provides a niche market which some growers may profitably fill. Another is the concern shown for animal welfare and the wish to ensure that the production methods used do not cause suffering. Governments have responded by the development of codes of practice which certify that the food has been produced in the manner claimed and by banning some practices which are thought to be incompatible with animal welfare. A recent example is the United Kingdom Register of Organic Food Standards.

1.1.5. Environmental goals. Agriculture is the principal land use over much of Europe. The appearance of the landscape and many other environmental features result from farming practices over many centuries. Modern

farming, using mechanical power and chemical inputs, has the capacity to alter this environment rapidly and sometimes in directions which are not desired. Agricultural policies have paid growing attention to some aspects of this development. These include:

Changes in the landscape, the loss of hedge-rows, the reclamation of land from forest and from wet lands and the disappearance of some long established grassland areas.

Pollution of water, including both water courses and underground aquifers. Some of this results from the misuse of chemicals such as pesticides and herbicides, some from the leaching of nitrates from intensively cultivated and fertilized land.

Loss of habitat for wild plants and animals. In some countries agricultural policy has had to adapt to allow for the protection of species which have become rare. Part of the concern is for the aesthetic value of a rich and diverse wild population. Part of it relates to the desirability to preserve genotypes which may, at some future data, prove to be of value to human populations.

Agricultural employment has been the basis of the cultural life of most rural communities. Its decline threatens not only the economic viability of some villages but also the survival of traditional customs and even of languages. In some senses the problem, may seen to be intensified when richer incoming populations force up the price of housing and introduce values characteristic of the affluent urban communities. Agricultural policy in such circumstances has come to be seen as part of a wider "rural development policy", changes in the EC structural policy during the 1980's illustrate this development. Three Commission policy papers passed to the Council in July 1988 represent the view of the Commission on the range of problems arising in rural society, they are as follows: Future of Rural Society, Com (88) 501; Communication on relations between agriculture and the Environment, Com (88) 338; Forestry Action Programme, Com (88) 255.

The main thrust of agricultural policy in Europe has related to farmers and production on the farm. The relationship between agricultural production and food production is complex and in Europe where more than 80% of food is sold in processed form, of great importance (SLATER, 1988). Food concerns have focussed on the safety and quality of food, and to some extent on the balance of power between the farming sector and the food processors and retailers. The underlying assumption seems to have been that provided the supply of food enabled consumers, at their prevailing income levels, to buy sufficient food, nutritional needs would be met. In this sense the contemporary concern with the content of the diet represents a new element in policy thinking which, if it is to be effective, must modify traditional approaches to policy making for agriculture and the food industries.

2. European agricultural policy — successes and failures

Agricultural policies in Europe represent a response to a wide range of different physical, economic and political circumstances. There is no space within this paper to describe in detail the arrangements which have existed in recent years. A recent FAO study (ALEXANDRATOS, 1990) provides an up to date account of the central features of agricultural policy in both the East and the West of Europe.

2.1. Successes and failures — Western Europe (The EC)

In Western Europe the Common Agricultural Policy (CAP), of the European Community (EC), has been the most important legislative intervention in agriculture. Some of its successes and failures are described below. For a fuller account of the policy see, for example FENNELL (1987). OECD policy studies provide a review of agricultural policy in other Western European countries.

The most important success of the CAP has been political. Without a common policy for agriculture several members of the Community would not have been willing to joint and the EC in its present sense could not have existed. A less discussed success, but one which has been important in the context of economic change in Europe, is the peaceful transformation of agriculture which has taken place during the past 40 years. Some 7.8 million people have left the industry which now accounts for only 6% of employment in the original member countries, compared with 16% in 1962 (Calculated from FENNEL, 1987).

These developments have taken place in an environment in which the productivity of the industry has greatly increased. In part this reflects the exodus of labour. In part it is due to the application of new technologies which have raised yields and reduced the labour needed for most farming activities. The Community as a result has enjoyed sustained increases in total output so that its goal of food security has been more than adequately fulfilled.

The extent to which these changes in the structure and output of agriculture are due to policy, or would have occurred anyway as a result of economic and technical progress is not clear. However, the CAP, in common with policies in many other Western European countries, provided both a stable and a relatively high price level for most important agricultural products. Within such an environment the risks attaching to investment have been reduced and the rewards greater than would have been the case in a free market. The role of structural policies which are specifically designed to encourage the modernization of farms, the consolidation of fragmented holdings, the movement of people to new industries or their early retirement seems

much less important. In much of the EC a major instrument of structural change has been part-time farming or "pluriactivity". Such systems enable the farm to continue as a business whilst the farm family enjoys an income considerably in excess of the profits it generates. The opportunities for part-time farming depend critically on developments in other economic sectors. The rate of structural change thus appears to have been more closely related to external economic factors such as the rate of economic growth, the level of unemployment and the demographic characteristics of the population.

For its critics the central failure of agricultural policy in Western Europe, as in many other developed countries, has been the economic waste associated with the production of surpluses. By keeping prices above market clearing levels production has continued despite the fact that the product generated has been less valued by consumers than the resources used. There are a diversity of symptoms of this disorder. First, in order to maintain prices policies have had to impede the working of international trade. By barriers to imports and subsides for exports production has been allowed to expand in high cost areas whilst farmers in countries with lower production costs have been forced to contract. Second such protection, although initially being effected through increased prices to consumers, has involved growing public expenditure. The results of this, in the EC, led during the 1980s to a series of reforms which were designed to contain the cost of the CAP to the EC budget. The introduction of quotas for milk and of "stabilisers" for most other products provide an indication that the budgetary costs of the policy had become intolerable.

In the EC and other West European countries agricultural policies have sought to raise the real level of income of farmers, particularly the smaller family farm. Prices have been sustained well above world clearing levels resulting in transfer payments to agriculture which have been estimated as 79.6 billion ECU for EEC 10. (OECD, Agricultural Policies Markets and Trade: Monitoring and Outlook 1988, Paris 1988, p. 58) CAP has been that their incomes have continued to lag behind those of other people. Price policy rewards most those who produce most, the large rather than the small farmer. The poorest farmers in Western Europe tend to be small and situated in remote, often inhospitable areas. For them price increases offer little benefit. Price policy also tends to create difficulties for new entrants to the industry. Higher prices tend to become capitalized into the value of land. HARVEY (1989) suggests that land prices in the United Kingdom are 46% higher as a result of current support policies in the EC. As a result the incomes of those who joint the industry, and have to pay this higher entrance fee, are depressed. Over a period of years inflation if it is reflected in agricultural prices, may lessen this difficulty for those who survive. If prices fall in real terms,

as has been the case in the 1980s, many of these newcomers to farming are among the most vulnerable, their greater technical knowledge and energy being insufficient to cope with the burden of debt.

2.2. Successes and failures — Eastern Europe

In Eastern Europe agriculture represents a much larger share of economic activity and employment than in the West, refer to Table 1. The economic implications of agricultural policy thus carry even greater weight in the overall welfare of the populations of these countries than they do in the West. In Eastern Europe and the USSR the growth rates of agricultural output, with the exception of the USSR in the 1970's, have been higher than in the West (Table 2).

This success in increasing output has been insufficient to prevent the countries concerned, as a group, becoming more dependent upon imports.

Table 1

Contribution of agriculture to the national economy

Country	Year	% Share of agriculture in	
		GDP/NMP	Employment
Bulgaria	1981–5	15.0	26.0
Czechoslovakia	1981–5	8.0	18.0
GDR	1981–5	8.0	13.0
Hungary	1981–4	20.0	26.0
Poland	1981–5	18.0	35.0
Romania	1981–5	16.0	34.0
USSR	1981–5	12.0	26.0
EC 12[a]	1988	3.4[b]	7.7

[a] CEC (1990).

[b] Gross value added/Gross domestic product NMP Net Material Product: GDP Gross Domestic Product NB: The figures for Eastern Europe cannot be compared directly to the EC 12 figures as they represent different indicators, they do however give some insight to the degree of significance agriculture has in the economies of the regions.

Source: ALEXANDRATOS (1990), p. 31.

Table 2

Growth rates of gross agricultural production

	1961–70	1970–80	1980–86
Western Europe	2.1	2.0	1.3
Eastern Europe	2.7	2.1	2.7
USSR	4.1	1.2	2.3

Source: ALEXANDRATOS (1990), p. 61.

Table 3

Per capita calorie consumption 1988

Country	Calories per capita per day
Bulgaria	3634
Czechoslovakia	3473
GDR	3800
Hungary	3541
Poland	3298
Romania	3358
E. Eur + USSR	3408
W. Eur	3383

Source: FAO Production Yearbook

In 1961/3 the USSR was 100% self-sufficient, by 1984/6 its self-sufficiency had fallen to 92%. It is disturbing that, despite the generally high availability of food in terms of calories per head, see Table 3, reports suggest that consumers in many Eastern European countries are still unable to buy the quantities of food they want. The existence of queues, reports of shortages and the key role played by food prices and agricultural production in much current debate suggest that one of the tests of recent reforms will be their success in creating a better balance between supply and demand. Several elements seem to be involved.

It is possible that these official figures overstate the availability and consumption of food. Figures in calories do not reveal the precise types or quality of foods available. The statistical procedures used may be unreliable. Losses in the food distribution system may not be fully accounted. President Gorbachev estimated that some 20% of USSR food production was lost in distribution (ALEXANDRATOS, 1990).

Agricultural production has focussed on quantity of output rather than quality. This may account for substantial wastage of food after it has been purchased. It also impedes opportunities for exports to countries in the rest of the world which would enable more efficient use to be made of the available resources.

Food balance sheets, upon which we rely for this information are an imperfect guide in assessing the nutritional status of a population. They provide evidence of the aggregate level of production and trade but they exclude questions which relate to wastage, quality and the distribution of food within the population. Given that current concerns are with the individual diets which in the long run lead to avoidable disease, lack of the distributional evidence of the deficiencies seriously weakens our ability to interpret the nutritional situation.

Shortages may be the result of inappropriate pricing policies. Food prices have been kept down in most Eastern European countries by means of very substantial subsides. It is, for example, estimated that food subsides represented some 13% of public expenditure in the German Democratic Republic. Since money incomes have been allowed to rise, and since there has been a shortage of many other consumer goods the demand for foodstuffs, especially meat has been stimulated. If food prices reflected fully the costs of production and consumers had access to a greater volume and variety of other goods, the quantity of meat which people sought to buy might well fall.

Quality deficiencies may not have impeded trade within COMECON where exchanges have occurred at prices determined outside the normal flow of world trade. However, transactions which do not reflect the costs of production may lead to a wastage of resources and weaken the ability of producers to invest in new technology and equipment. Such trade may account for the fact that some Eastern European countries which themselves appear to be self-sufficient have experienced shortages of food. Had the food been paid for at world prices it could have been replaced by imports of at least equal value. Because the countries which produced it have been made poorer.

It is misleading to describe these problems as if the situation in the food and agricultural industries of all Eastern European countries were identical. There exist very important differences in the development of food processing and distributive industries as well as agriculture. Such differences are likely to play a role in determining how best to cope with the problems of shortage, of quality and pricing.

3. New influences on agricultural policy

The desire to change existing agricultural policies stems not only from the failures of past arrangements but also from some new developments outside agriculture itself. This section of this paper considers some of these issues.

Increased production in the developed market economies of the world led to an increase in the degree of agricultural protection. This is a matter of concern for low cost agricultural exporters who find their markets lost to the subsidised output of higher cost producers. The reform of agricultural trade has become one of the focal points of the current round of negotiations in GATT (The General Agreement on Tariffs and Trade). Many other elements are involved in this debate which is not expected to end until December 1990. If the negotiations succeed the level of agricultural protection will be reduced. The EC has already proposed a reduction of 30% between 1986 and 1996. Any such move will intensify the adjustment problems facing high cost producers and their governments.

The obstacles to agreement remain substantial and it is unlikely that progress towards a more liberal trading system will be rapid however, restrictions on export subsidies, which are early targets for the reformers would imply an immediate need to reduce production within the EC.

In the longer term it is possible that the countries of Eastern Europe might fully participate within GATT. Should they do so, and assuming that agricultural trade is liberalized, new opportunities for trade within Europe will develop. Initially it might seem that the existence of shortages in the East and surpluses in the West provide a convenient match. However, given the natural resource base and the relative stage of economic development of most Eastern European countries, the long run flow of trade may add to the problems of EC agriculture. If, as is hoped by reformers, market forces stimulate production and lead to improvements in quality of agricultural goods in the East, possibly with the aid of imported capital in the processing and distributive industries, their market may have to be in the West. Revenues resulting from such expansion could provide one source of funds for the import of consumer goods. Such trade is in the economic interest of Europe as a whole but it may further constrain the opportunities for Community farmers in their "own" market. Even in the short run, if European trade were liberalized and exchange rates free to move, price differences might lead to a substantial flow of goods from East to West.

The barriers to a freer trading system are not simply through economic instruments. Governments may use sanitary and phytosanitary regulations to ban the import of agricultural products. One element in the current GATT debate, which will have a significant impact on the success of any agreement reached in actually promoting freer trade, is the negotiation of an agreed framework for the operation of such health based regulations for application by all countries. Work done by FAO in the development of a revised Codex Alimentarius provides a basis for progress in freeing trade whilst ensuring that high standards are observed in protecting consumers and the agricultural industries of importing countries from the risks of disease.

Agricultural policy is likely to be effected by measures taken to counter the "greenhouse effect", a process of global warming which is believed likely to result from the discharge into the atmosphere of gases such as CO_2 which result from modern industrial activity. Several elements are involved. As a user of fossil fuels, both directly on the farm and indirectly in the inputs of chemicals and machines it employs, agriculture contributes to the release of "greenhouse gases". Since plants absorb CO_2 they can act as a carbon "sink" reducing the tendency to global warming. Annual crops simply recycle such carbon but tree crops which may last for several decades represent a longer term means of improving the environment which has clear consequences

for land use. Ruminant animals, as a result of their digestive processes, release a considerable quantity of the "Greenhouse gases". Any reduction in livestock production, would from this point of view, be advantageous. Much more important would be policies designed to limit or slow down the "greenhouse effect', for example by reducing the use of fossil fuels. These would affect both the production costs of farmers and their supply industries and the real income of consumers. Agricultural policy would have to take account of such changes.

Global warming is an international environmental concern. Agricultural policy will also have to take more note of local environmental issues. Within the EC limits on the use of nitrogenous fertilisers, controls on pesticides and herbicides may be expected to be intensified. Such changes raise farmers' costs and, if their competitors are not similarly restricted, reduce their ability to compete. The urban population, at least in Western Europe, seems likely to make increased demands on rural resources and place constraints on the way in which they are used by farmers. Part of this may be through the use of more land for housing, roads and leisure activities associated with urban lifestyles. Part may relate to ensuring access to the countryside for visitors whose requirements may include the preservation of particular landscapes or species of wild plants. Agricultural policy will have to take this into account, not least because the detailed arrangements are likely to have to vary considerably from place to place. Already the Community has made provision for some developments in this direction through the creation of "Environmentally Sensitive Areas".

Modern intensive agriculture makes use of non-renewable resources, notably oil, which have greatly increased its productivity. Concerns are now growing about the sustainability of such a system. The concept of sustainability is complex and often ill defined. In the sense that any human system can be infinitely durable it is clearly misguided. However, the notion that current generation should not lightly pre-empt the options open to their more immediate successors is widely respected. At this level some of the features of contemporary agriculture do give cause for concern. In Europe this focusses less on problems of soil erosion, which are evident elsewhere in the world, and more on the wastage of non-renewables, either in the form of surplus production or through the use of techniques which are unnecessarily resource intensive. Debate on these issues is at an early stage but it is difficult to believe that it will not ultimately impose changes on agriculture policies and practice in the direction of farming systems which are less intensive and make fuller use of solar rather than fossil energy.

Agricultural policy has been largely conceived in terms of the actions and interests of farmers. This approach is increasingly inadequate in an economy in which most of the food consumers buy is highly processed and distrib-

uted through powerful retail organisations. These food industries are affected by agricultural policies. For example, the CAP ensures that ingredients used by EC food processors cost more than they would if access to world markets were not restricted. Food manufacturers are only able to compete in third markets if the Community provides an offseting subsidy to eliminate the effect of the CAP on input prices. The calculation of such subsidies is critical to the success of such food exporting firms.

Developments within the food industries affect the opportunities for farmers. The growing concentration of power among major retailers raises issues of competition policy which affect farmers as well as consumers. Development in agricultural policy may be needed to offset such monopolistic developments and to strengthen the bargaining position of farmers if markets are to function satisfactorily.

In the European Community the creation of a Single Market is intended to be completed by 1992. This is an ambitious task and some impediments to trade may survive into later years. However, substantial progress has been made and it is not without importance for the agricultural policy maker. One direct result has been an agreement to remove the system of monetary compensatory amounts (MCAs) which have become a feature of intra-EC agricultural trade. MCAs have been used to avoid the necessity to adjust the internal agricultural support prices within member countries following a change in their exchange rates. Countries whose currencies appreciate would, in the absence of MCAs have to reduce, in terms of their own currency, prices which have been fixed in the common unit of account, the ECU. Similarly countries with currencies which lose value would have had to put support prices up in terms of their own currency. The removal of the MCA system will expose farmers in strong currency countries to competition from other member countries. The result is likely to be pressure either to raise common prices, a process which makes the control of production even more difficult, or to provide special aids for such farmers on a temporary or even permanent basis. The creation of a Single Market must also be expected to have consequences for the location and sourcing policies of food processing companies. In the past it has been necessary to have plants in several countries in order to accommodate the varying restrictions they have applied to the operations of food processing and trade, given an assurance that products can be moved freely within the whole of the Community it is likely to be more attractive to build plants of optimum scale where the location of raw materials and markets is most attractive.

In considering future possibilities for agricultural policy these new issues have to be added to the unresolved problems of past policies, the inappropriate levels of production in both the East and West, the difficulty of making changes which do not have undesired consequences for the incomes of farmers

in the West and consumers in the East and the failure of agricultural policy, as it has been practiced, to sustain the economies of many rural areas which are predominately agricultural in character.

4. The implications of nutritional guidelines for agricultural policy

A first nutritional requirement is that the agricultural and food industries supply reliable and safe food at prices which all consumers can afford. Policy can contribute to this by ensuring that the quantity of food available is sufficient and by establishing and enforcing standards that prevent unsafe food from reaching the consumer. In aggregate, in Europe, there is no problem about availability but this does not mean that every person is able to afford a satisfactory diet. Differences in income distribution mean that at the lowest level of income food supply remains a serious problem. Large families on low incomes, pensioners with few private resources and those who become unemployed spend much larger shares of their incomes on food and high food prices limit the choices open to them. For those who fall through the net of social security provision food supply may become a matter of scavenging even in the affluent countries of Western Europe. Such deprivation cannot be remedied by nutrition policies or agricultural policies, its removal is much more the responsibility of social policies and macro-economic management. However, agricultural policies which raise the price of food do exacerbate the problem.

In Eastern Europe changes in price policy maybe needed to ensure that demand and supply for particular products are brought in to harmony. Such changes present special difficulties for those whose incomes are low and for whom cheap food has been one of the main benefits of a planned economy. If the social consequences of a move to a more market determined price policy are to be acceptable, measures to distribute income towards those who earn least are likely to be essential. As in the West success in resolving these challenges will depend more on macro-economic progress than on agricultural policy alone, but because of the larger share of agriculture in the economy the contribution which a more efficient food and farming sector might make is likely to be of greater importance.

There is no ground for complacency in Europe about the safety of food. Recent statistics show that there has been, in the United Kingdom, for example, a rapid increase in the incidence of food borne disease.

In England and Wales the Central Public Health Laboratory Service at Colindale regularly publishes statistics on food born disease. Their studies show that reported cases of food poisoning caused by Salmonella and by *Campylobacter Jejuni* have increased rapidly over the last four years (WAITES,

W. M., 1990). Infections from Salmonella, Listeria and Campylobacter have all increased. Many of these may result in minor illness from which the sufferer rapidly recovers. Some, however, have much more serious results for vulnerable individuals, the old, the infirm and pregnant mothers. Such infections impose an economic cost in the form of lost days at work, medical and hospital requirements and impaired efficiency even among those whose illnesses may not require medical treatment. Although food borne disease may still account for relatively few fatalities compared with motor car accidents or the consequences of smoking, it is a serious and growing cause of concern for the food and agricultural industry as well as for nutritionists and health workers.

From the industry's point of view, where breakdowns have occurred in food safety, there has been a damaging and costly loss of confidence in their products. The recent publicity given to egg borne salmonella infections in the UK, for example, resulted in severe losses for egg packers. Following such breakdowns debate continues among the most informed experts about the extent of risk facing the consumer and about the most appropriate way to reduce it. The food industry may find it difficult to restore a level of confidence which corresponds to the objectively measured risks associated with its products.

Restoring confidence and increasing understanding among the public is thus in the interest of all sectors of the food industry from farmers to food retailers.

Nutrition has become a concern of agricultural policy because evidence has linked diet with diseases which are responsible for large numbers of deaths. Good nutrition is a necessary but not a sufficient condition for health. Many other aspects of the way people live and work are also of importance. Nutritional considerations have therefore to be balanced, by policy makers, with other health concerns as well as with the implications for other sectors of the economy of the changes in diet they imply. Agricultural resistance to proposals which threaten the livelihoods of many farmers is likely to be a significant factor in the evaluation of alternatives by politicians. In Norway, for example, where an early start has been made on the development of nutritional policy, agricultural anxieties have tended to impede its application. (See for example C. Blythe, Norvegian Nutrition and Food Policy, in: Food Policy, August 1978 p. 163, IPC Business Press.)

An object of nutrition policy must be to help consumers take informed decisions about what they eat. Given assurance that the foods available are safe the purpose must relate to the choice of foods which together will constitute a healthy diet. In this context a healthy diet means one which is appropriate to the individual. This will change as people age and as their lifestyle alters. The total quantity of food needed will decline as growth ceases

and physical activity declines. The mix of food for most people in North Western and East Europe needs to include less fat, sugar, salt and alcohol and more fibre. However, there are no universal recipes right for each person.

Consumers have to select foods on the basis of the information which is available and their understanding of its significance. The temptation to describe some foods as "good" and others as "bad" needs to be resisted. An excess of a "good" food may be more damaging than modest proportions of "bad" foods.

Labelling may enable consumers to consider the contribution of processed and packaged foods to their daily diet. However a certain level of understanding of nutritional requirements is necessary to relate this information to personal needs. In the absence of such understanding we may have absurd results such as the consumer who chooses, on health grounds, to opt for skimmed milk during the week and then celebrates at the weekend by adding cream to his meals.

A potentially important source of improved diets in Northern Europe is the substitution of a greater proportion of fish for red meat in the diet. One of the features which make mediterranean diets superior, in terms of current nutritional insights, is the relatively high proportion of fish in the diet. The ability to increase fish production is constrained by the ability of the fish population to make good stock losses due to the fishing industry. At the moment most fish in Northern Europe is used for the production of fish meal, as a high protein addition to the diet of farm animals, rather than directly for human food. Consumer tastes limit the types of fish which are consumed. The development of the aquaculture industry may help to make available larger quantities of the preferred sorts of fish. Already a substantial proportion of salmon production comes from "fish farms" and the expansion of this industry, despite its economic and technical risks, represents a means of making available more food with desirable nutritional characteristics. If this is to happen, it is essential that consumer demand grows at a pace which will support extra output. If it does not price collapses will mean that investments in aquaculture prove unprofitable and this potential source of nutritionally attractive food remains a small proportion of the total food output.

One result of a loss of confidence in "orthodox" food among some relatively affluent consumers has been the growth of a market in so called "health foods". In nutritional terms there seems little to choose between food which is grown without the use of "artificial" chemicals and that produced according to conventional methods. More alarming is the possibility that the concern for healthy food may make some ill informed consumers vulnerable to spurious claims for exotic products which have no special merit and may cause some harm. There is a need for the dispassionate, scientific testing of such products and the provision of accurate information to consumers.

An improvement in diet in terms of its composition would have substantial consequences for the sales of foodstuffs in Europe. The effect of European agriculture will depend upon its ability to compete with the rest of the world. The availability of food is a precondition for consumption but this does not mean that because food is produced in a particular area it will necessarily be consumed in the same place. In an open market goods will be produced where costs are lower and consumed where prices are highest. A reduction in the consumption of animal products in Europe need not therefore lead to a contraction of the European livestock industry, provided it is competitive in world terms. Equally a regulation which limited milk or meat production within Europe would do nothing to restrict consumption if these products came in from abroad, attracted by the high prices shortages would create.

The problems which would arise from reduced consumption of animal products in Europe stem not simply from the loss of markets at home but from the non-competitive nature of much of its agricultural production. They are very formidable. Livestock and livestock products account for some 51% of total value of farm output in the EC (calculated from CEC, 1989), a cut of 20% in production of these commodities would displace a very substantial share of agricultural resources. It is estimated that it takes double the area to feed one person a conventional diet (i.e. meat inclusive) as compared with a vegetarian diet. The vegan diet offers the most economical usage of land requiring below one quarter of the land needed to provide for the conventional diet (BIRCH et al., 1990). Assuming because costs were high that, in a free trade environment, there would be no scope for expanding exports, contraction of livestock output on such a scale would have a major impact on agriculture as a whole. Excess land and unsalable feedingstuffs would depress the prices of arable and vegetable crops and, at least in transition, the residual market for livestock products would be chronically over-supplied.

In Eastern Europe the position is rather different. A reduction in the consumption of animal fats would release resources and tend to bring markets into equilibrium. However, agriculture in Eastern Europe would face additional challenges in responding to an increased demand for high quality fruit and vegetables. Additional supplies may be imported. Balance of payments concerns may make this difficult and to provide supplies from domestic resources will require new techniques at the farm level, a new approach to the marketing and preservation of vegetable products and the development of changed attitudes to the preparation of food in the domestic kitchen and the catering establishments.

Changes in European diets would have important implications for other countries in the world. Existing policies and consumption patterns have contributed to a situation in which world markets have often been depressed as a result of the disposal of agricultural surpluses from the EC. A reduction in

livestock consumption would cut the volume of animal feedingstuffs used within the Community, quantities of cereals and cereal substitutes currently used for this purpose would no longer be needed. The immediate consequence might be further to increase the budgetary cost of the CAP and lower world cereal prices. If as seems likely the industry contracts, reduced use of animal feedingstuffs would affect countries overseas who supply cereals, cereal substitutes and high protein feeds. Some developing countries will lose markets for products such as cassava and oilseeds. For the United States there would be a cut in sales of soya, maize gluten as well as downward pressure on the coarse grains market as a whole. The feedingstuffs industry which employs significant numbers of people would be forced to contract both in Europe and elsewhere. The application of a nutritional policy which had such results, is thus likely to be resisted by lobbyists not only for European farmers but for some developing countries and the major international grain traders.

The world market is not confined to cereals. The shifts in diet which are now being recommended on nutritional grounds include increased consumption, in many parts of Europe, of fruits and vegetables. There is no reason to doubt the ability of European agriculture, as a whole, to produce extra quantities of those products which are suitable to the climate of the region. However, there seems likely to be a growing market for exotic fruits and vegetables in order to provide greater variety in the diet. For some countries situated in tropical and sub-tropical regions this could offer an important market opportunity. It is tempting to think of this in relation to the need of many third world countries to generate export revenue, however, the location of production is likely to be determined by market characteristics, quality control, the existence of a good transport infra-structure and the availability of capital to finance expansion. In these respects poorer countries are at a disadvantage.

Current thinking about agricultural policy, the need for a lower level of protection if the GATT round is to succeed and the need to target support more accurately towards social and environmental goals suggests that any further increase in the budgetary cost of commodity support would simply accelerate change in the way the CAP operates. Such changes would seek to eliminate surpluses and so would tend to strengthen rather than weaken world prices. For agricultural exporters, including those in developing countries, this may be good news. For the increasing number of poor countries who have become net importers of food the longer run consequence might be added problems both with their foreign exchange balance and with the supply of food for their people.

5. Where should policy go now?

The intention of nutritional guidelines currently being proposed is to alter the diet of most people in Europe. In practice this has to be attempted in ways which are consistent with other goals of food and agricultural policies and in the context of some dramatic changes which this industry now confronts. Nutritional goals are more likely to become an explicit part of policy if they can be seen as part of a general strategy for the European food and agricultural sector and not simply as an attack upon the existing agricultural interests.

New policies have to operate within a changing economic and political environment. The agricultural and food sector is already exposed to a variety of changes apart from those which might emerge from a nutritional policy. The growing level of real income in much of Western Europe has diverse implications for this sector. Greater concern for the environment, stricter measures to control pollution and the need to provide resources for non-agricultural uses in housing, transport and leisure may all raise costs of production so far as farmers are concerned. The greater wealth of the population, combined with demographic changes which increase the proportion of old people in the community will lead to heavier demands for health services and new opportunities to supply niche markets for the food industry. In Eastern Europe the move to a market economy implies much more dramatic changes for both agriculture and the food industries. Whilst the need to change dietary habits is common to both West and East the process of change is likely to be difficult and uneven.

The ability of an industry to adjust to a change in its economic environment is enhanced if it already uses resources competitively. If it does not the proportionate shift in resource use which may be required is likely to be larger and more difficult to accomplish. Several pressures are forcing agriculture in Western Europe towards a more competitive basis. The prospect of greater openness to the world market implies that EC agriculture will have to adjust to overall trends in world price levels. A shift in agricultural resources from food production to environmental protection provides an opportunity to make the food production industry competitive by drawing a clearer line between the social purposes of much agricultural policy and the need to provide a secure supply of food. The introduction of new techniques based on biotechnology may tend to favour the more sophisticated and capital rich agricultures rather than traditional family farms. If European agriculture wants to make full use of its natural resources and geographical advantage it may have to accept the structural change such developments imply. If such an industry is to develop it will be necessary to provide compensation for those most adversely affected. Part of this may come from payments to

farmers to pursue environmental priorities. Part may be in the form of early retirement payments and assistance for retraining. Some may have to take the form of more continuing income support on the grounds that there is no reasonable alternative way by which the farmers concerned can be expected to earn an acceptable income in other activities. However the key to rapid change is likely to be the bouyancy of the European economy as a whole.

Nutritional policy, which also implies change in the farming sector, might be made less attractive in the short and medium term by changes in farm policy which stemmed from the need to bring EC prices closer to world levels. The politically acceptable rate of change is unlikely to be rapid and in the interim supply control will be made more difficult if nutritional guidelines lead to a significant cut in the consumption of milk and meat. It will be tempting for policy makers to resort to quotas which seem to offer a relatively quick and certain solution to the need to contain output. Such policies sustain a non-competitive industry and freeze the pattern of production. For new entrants their effect is to raise costs so that the burden of moving, at any later date to a more competitive industry is increased. Such inflexibility, with its implicit imperative to compensate farmers should markets decline in the future as a result of nutritionally inspired changes in diet might make such policies very unattractive. Thus, whilst supporting the goal of a healthier diet, it might be sensible for the nutritional policy maker also to seek an industry which becomes better able to compete in the world and which uses prices rather than physical controls to bring total supply into harmony with consumption.

In incorporatig nutritional considerations into agricultural policy making in Eastern Europe it is impossible to separate the future of agriculture from the development of the economies concerned as a whole. The first task must be to empower the consumer to make nutritionally sound choices. At the root of this must be an increase in the level of real income at the disposal on consumers. Whilst improvements in agriculture may have an important part to play in economic progress much of the change will depend on developments in other parts of the economy. Given higher levels of income it would be helpful to redistribute existing food subsidies to those who are poorest and allow food prices to rise. If food prices reflected more accurately the costs of production consumers would probably shift their purchases towards goods which seem to be more in line with nutritional advice, to fruit and vegetables rather than animal fats. Such a shift, provided resources were free to move would provide its own rewards to those who produced these goods. It would also probably result in a healthier diet at lower resource cost.

An issue which has to be confronted is the use of price as an instrument to steer consumption in a nutritionally appropriate direction. In many countries heavy taxes are placed on the consumption of tobacco and alcohol in part

at least to deter consumption. They have not been very effective because demand proves to be very price inelastic but they have become an important source of government revenue. In the EC the CAP has made milk relatively expensive compared with vegetable oils, as a result margarine is made more competitive with butter. It seems that consumers are resistant to price shifts which affect whole categories of goods but may be willing to change their consumption patterns if the price of close substitutes alters. Recent trends, for example, the falling real price of poultry and pig meat in comparison with beef and sheepmeat have encouraged consumers to adjust their consumption patterns towards the cheaper commodity. Even where consumers are reluctant to change their eating habits the processing industry may be much more sensitive to relatively small price shifts. The need to remain competitive and the opportunity to substitute ingredients may make their response more positive than private consumers or caterers. Taxes imposed on "less healthy foods" need not be inconsistent with free trade provided foreign producers have access to the market on the same terms as domestic farmers. A tax could be placed on products such as milk, sugar or beef which could be used, for example, to compensate farmers forced to adjust or devoted to funding education relating to health and nutrition.

Perhaps the most important step in incorporating nutritional considerations into agricultural policy is to re-think this policy in terms of the "Food Chain" as a whole rather than consider agriculture by itself. In terms of the variety and quality of the food eaten, the advertisments wich play an important part in initiating changes in consumption patterns and the efficiency with which resources are used by the industries beyond the farm gate play a larger part than do farmers themselves. A food policy has then to include these enterprises within its remit and to provide incentives which encourage processors and retailers to offer consumers food products which enable them to make informed choices. Such an approach is also in the interests of agriculture, if it is to be exposed to a market without price support. By ensuring that the quality of food is maintained at every level, by the application of standards of food hygiene which prevent outbreaks of food poisoning and through ensuring that goods entering from other countries do not fall below these standards the governments and industries of Europe share a common interest in retaining domestic markets and enhancing their competitive strength in markets in other countries.

Nutritional policy raises issues which cross the existing frontiers of ministries in most countries. In the United Kingdom, for example, they concern the Department of Health and Social Security, the Department of Trade and Industry, the Department of Education and Science as well as the Ministry of Agriculture, Fisheries and Food. In each of these departments nutrition is one issue which must compete for attention with many other claims.

There exists concern that in this process the nutritional imperatives are diluted in order to accommodate other interests. Such anxiety leads to demands for a separate institutional structure for policy relating to nutriton. The appropriateness of such a move depends on the view adopted concerning the role of government. Where ministeries are seen as advocates for client interest, separate representation is important. Where, in contrast, the ministries are seen merely as the mechanisms through which government reaches a view on the national interest as a whole, the representation aspect of policy formation may more properly be left to external pressure groups. Within such a system pressure groups may interact with several ministries each of which reflects their input in a general area of policy to which it relates. The creation of a separate "nutritional" ministry might, on this model weaken the influence of nutritional thinking, since the expertise would be divorced from the situations in which the decisions about action have to be taken. There is no reason to believe that a single model is appropriate for every country or, even within countries, that the same solution will always be appropriate.

The application of nutritional advice depends, in a free society, on the voluntary action of individual consumers. Consumers need both a sound education in matters relating to health and nutrition and accurate, understandable information about the foods they are offered. This is another area in which the interests of governments and the industry co-incide. The priority given to nutritional and health matters in education depends largely on governments. However, the food industry and the media may have an important role to play in building on the foundation acquired in childhood. During the life of most people many new food products are likely to be offered to them. If they are to choose wisely their education needs to be brought up to date. Such information may be provided by food companies and feature in television and press coverage. One of the difficulties is the lack of confidence among consumers about the information they receive. A code of practice within food industries and the press might help to avoid the sort of sensational reporting or misleading claims which have weakened consumer trust in the past.

Given better understanding consumers still need information about the content of the foods they are offered if they are to make rational choices. This is less straightforward than might appear. Without better understanding the information given may be useless. Without available information consumers have no incentive to develop their understanding, indeed such understanding as they have may be misapplied on unreliable information or assumptions. To fully regulate diet consumers need information about everything they eat. Labelling is, however, only possible with processed foods so that an important part of the diet may not be covered. For the food industry the provision of such information may seem costly and unrewarding since at most only a minority of consumers will actually make use of it. Despite this and partly to create

confidence the food industry as a whole must be prepared to provide information not only about the contents of the food but probably, too, about the way it has been produced. Although this may include nutritional information the industry's motive is not primarily one of nutritional policy but rather of making sure that the agricultural and food industries of Europe enhance their ability to compete both in their home market and, where appropriate, in markets elsewhere in the world.

In the market economies which now seem likely to dominate Eastern as well as Western Europe industries have to respond to the demands of consumers. The food and agricultural industries have in the recent past been shielded by agricultural policies, in the West and by food subsidies in the East. In both regions there now seems to be a willingness to expose these industries to the discipline of the market. In such a market food consumption is likely to be influenced more by the enjoyment, social as well as individual, which people derive from eating than by any physiological needs. If this diet needs to be changed individual consumers have to discover more satisfaction in the reformed diet than in their existing patterns of consumption. In changing such attitudes direction from official agencies has a limited role, much more depends upon persuasion and the provision of alternatives which are not only more healthy but also more attractive than the existing diets. Once the food industry is convinced that consumers will buy products which make up improved diets it is likely to respond very rapidly, using the skills of the modern food processing and packaging sector to offer new products which combine enjoyment with better health characteristics. This will require little intervention from the State, beyond ensuring that misleading claims are not made. If diets are to be reformed there is thus a need for co-operation between the agencies responsible for health education and the food industries in providing both a message that change is needed and the means to make it in a relatively painless manner. For agriculture in Europe such changes will be super-imposed on others which are already uncomfortable. The farming industry which remains will probably be smaller and more competitive in global terms. It may also find an increased part of its revenues derive not from production of food but through the provision of services which enhance the environment and facilitate access to the countryside for a more affluent and healthy population.

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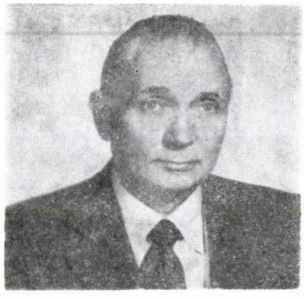
IN MEMORIAM



The Editorial Board regrets to announce the death on 17 October 1991 of Prof. Konstantin Vukov, one of its members, professor at the University of Horticulture, Faculty of the Preserving Industry, Department of Preserving Technology. Professor Vukov was a well-known expert in food science and technology with the special field of the physical, chemical and biochemical properties of sugarbeets.

Professor Vukov was born in 1920 in Törökbecse, Hungary (now belonging to Yugoslavia as Novi Bečej). After graduating in 1938 he studied at the Technical University of Belgrade until 1941 and continued his studies at the Budapest Technical University where he was awarded the degree of Chemical Engineer in 1943. His working carreer was started in the Kaposvár Sugar Factory as a chemist for the associated Helios Cannery. In this period of 1943–1951 he started his scientific activities, too, having made investigations on the chemistry of sweet sorghum and tomatoes. The results were put down in his first publication in 1947. In this time he also reported on the electrochemical properties of sugarbeet juices.

The next period, 1951–1975 bound him together with the Research Institute of the Sugar Industry in Budapest. During the first eight years he was in charge of the Department of Raw Materials, in the following six years he headed the Department of Technology.

In 1975 he was appointed professor at the University of Horticulture, Department of Canning Technology. In the frame of comprehensive investigations of the physical, chemical and biochemical properties of sugarbeets he developed measuring methods for the cutting resistance and the elasticity of sugarbeets.

As a result of his work in this field he prepared a dissertation on "The Physical and Chemical Properties of Sugarbeets" by which he got the degree of Candidate of Chemical Sciences (Ph. D.) at the Hungarian Academy of Sciences and later a degree of Doctor of Technology from the Budapest Technical University.

Acta Alimentaria 20, 1991
Akadémiai Kiadó, Budapest

His field of interest was extended to the fields of of the purification of sugarbeet raw juice and of the thermal stability of sucrose. The results were summarized in a dissertation on "The Hydrolysis of Sucrose and the Decomposition of its Hydrolytic Products" which led to his Doctor degree by the Hungarian Academy of Sciences (Doctor of Chemical Sciences, D. Sc.).

Professor Vukov was involved in a lot of scientific associations. Among these he was, since 1949, a member of the Hungarian Society for Food Industry, took part in the work of the Complex Committee on Food Science of the Hungarian Academy of Sciences; was elected by the C. I. T. S. to membership in its Scientific Committee. He was a member of the Editorial Board of the Journal "Cukoripar" (Sugar Industry) and of Acta Alimentaria.

The number of his scientific publications can be estimated 170. These have appeared mainly in Hungarian periodicals and journals but in a number of journals abroad, too.

The Hungarian Akadémiai Kiadó edited his book on "Physik und Chemie der Zuckerrübe als Grundlage der Vearbeitungsverfahren" (1972) which was translated and also edited in Turkey. The book on "Physics and Chemistry of Sugarbeet in Sugar Manufacture" was a joint edition of Hungarian Akadémiai Kiadó and Elsevier Scientific Publishing Co. (Amsterdam), 1977.

His successful research and educational work was acknowledged by the several honours and awards.

The modest and disciplined way of life he conducted made him rather popular among colleagues and students. He always was ready to help all who turned to him and encouraged his co-workers for deeper and more active scientific work.

His memory should be kept and may be put as an example to the generations of researchers to come.

I. VARSÁNYI

BOOK REVIEWS

Food technology for a dynamic marketplace

M. KROGER and A. FREED (Eds)

"Changing Food Technology" series, Vol. 2. Technomic Publishing Co., Inc., Lancaster, Basel, 1990, 208 pages

The editors selected papers from the Fifth Eastern Food Science & Technology Conference which was held in 1987 in Hershey, Pennsylvania. According to the conference program this publication is devided into four parts: Nutrition Marketing and Communications; Safety, Quality, and Regulatory Affairs; Ingredient Technology and Product Quality Improvements through Process Optimization.

Nutrition marketing is a relatively new field: Today's consumers are actively involved in finding out what nutrition can do for their long-term health. As a result, they want to know how foods they see on the supermarket shelf contributes to health.

Advertising and public relations are important nutrition communication tools, they influence opinion and motivate behaviour.

Hazard Analysis Critical Control Point (HACCP) program is very simply a technique for identifying problems before they occur. Components of HACCP include: assessment of hazards associated with producing, processing, storing, and/or preparing foods or with operation within an establishment; determination of critical control points necessary to control any identified hazards; and establishment of procedures to monitor these critical control points. HACCP has been used successfully since the early 1970s by the food processing industry.

Among food ingredients new sweeteners are in focus, brief review is given of acesulfame-k and alimate, the primary focus is placed on sucralose a stable, high-quality, nonnutritive sweetener.

Part IV discusses problem influencing refrigerated food quality and safety, possibilities given by controlled/modified atmosphere storage to extend shelf life and quality of fresh produce, and optimal trends in storage conditions for maximum retention of frozen foods quality.

The selected papers present the food scientist, technologist with current applicable information in the fields of marketing, nutrition, quality control and regulatory compliance, as well as direct applied technology, and will help to understand the relationship of these interdisciplinary fields.

ANNA HALÁSZ

Food technology: a view of the future

A. FREED (Ed.)

"Changing Food Technology" series, Vol. 3. Technomic Publishing Co., Inc., Lancaster, Basel, 1990, 134 pages

The booklet contains the following papers selected from those presented at the Sixth Eastern Food Science and Technology Conference held on October 1–4, 1989, at Hershey, Pennsylvania:

Profits in Responding to Consumer Concerns (H. B. Brown),

Health Topics of Concern — Diet and Health: Keeping a Balanced Perspective (F. H. Seligson),

Educational Needs and Trends in the Future — What Will Be Emphasized in in Food Science Education? (O. R. Fennema) (Summary only),

Food Science 2000 (D. B. Lund),
Aseptic Processing — Use of Ohmic Heating (D. F. Dinnage),
New Applications of Membranes in Food Processing (J. L. Short),
Monitoring of Product Quality Using Time–Temperature Indicators (S. C. Fields),
Microwave Packaging at Campbell Soup Company (M. L. Druin),
The Food Industry in The 1990's (A. S. Clausi),
The Future as Seen by a Government Administrator (R. J. Ronk, P. Thompson and K. L. Carson),
The Information Matrix (M. A. Boskovic),
Information Technology in an Electronic Age (C. C. Mundy and J. Donahoe),
Computer Technology in the Future of Food Manufacturing (A. A. Teixeira).

Although the relatively short papers address the above topics mainly from U. S. viewpoints, these brief reviews on consumer concerns, health topics, new processing-, packaging-, information- and computer-technologies provide a thought-provoking interdisciplinary overview on present trends and future of food science and technology in the food industry. The book is of interest to managers and technologists working in the food processing industries, and to professionals involved in educational programs in food science and technology.

J. FARKAS

Packaging foods with plastics

W. A. JENKINS and J. P. HARBINGTON (Eds)

Technomic Publishing Company, Inc. Pennsylvania, 1991, 326 pages

The book gives a wide-range survey on the production, characteristics and application of plastic packaging materials and containers. Each chapter contains well-arranged figures; the comparison of data is promoted by tables. At the end of nearly each chapter the main literary references are given about the topic. A glossary and an index chapter promote the better handling of the book and the unambiguous understanding of technical terms and abbreviations.

Apart from the preface the book is divided into 22 chapters. The introduction is on plastics revolution, it is followed by plastics and laminated plastics and their fabrication. The third chapter is on the physical and physico-chemical properties of plastics and then homo- and copolymers of various chemical structures that are utilized for different packaging purposes are dealt with.

Chapter five describes the filling and closing machinery of liquid foods and the wrapping and bundling solutions of solid foods. The chapter is completed with printing, coding and labelling part and future considerations are also outlined.

Chapter six is on food packaging and distribution industry and a special part is devoted to retail food stores.

Chapters seven, eight and nine discuss the problems of various food packaging, like fresh meat and poultry, cooked and processed meat and poultry and fish.

Chapter ten is about bag-in-box packaging.

Chapter eleven introduces the packaging of dairy products (milk, cheese and other products), chapter twelve of various liquids, (alcohol free and alcoholic and water), chapter thirteen the packaging of coffee, chapter fourteen of snack foods. Chapter fifteen discusses the packaging of low water content foods as cereals, baking mixes, rice, pasta, dry soup mixes.

Chapter sixteen deals with the packaging of baked goods. This large food category comprises packaged bread, rolls, yeast-raised sweet goods, cakes and pies, and frozen and refrigerated baked goods.

Cooking oil chapter gives the package requirements and package construction.

Condiments, spreads and spices chapter discusses the problems of squeeze bottles, mustard, ketchup and spicy sauces, syrups, liquid salad dressings, sugar, peanut butter.

Chapter nineteen is about the packaging solutions of fresh produce.

Chapter twenty surveys the packaging of pre-cooked dinners and also other packaging methods which promote their usage and require special heating solutions like microwave oven heating.

Chapter twenty-one studies the packaging questions of baby food and pet food, and chapter twenty-two discusses environmental matters and mentions biodegradable modern packaging solutions.

The book gathered the most up-to-date techniques of packaging with special regard to the connections between packed food and the packaging material. Regarding the thematics and the mode of presentation this is an especially useful work to read for packaging experts but also for those working on the field of packaging development and in education.

I. VARSÁNYI

Food additive user's handbook

J. SMITH (Ed.)

Blackie and Son Ltd., Glasgow, 1991, 286 pages

The handbook includes an overview of the use and consumption of food additives and gives many information on the safety evaluation of food additives. The book is divided into fifteen chapters, each of which covers a category of food additives.

Each chapter may be considered an individual study. The antioxidants chapter deals with promoters of oxidation by antioxidants. Detailed information is given on the major antioxidants, their properties, their application in different foods and on the regulations governing the usage of antioxidants in various countries. 33 tables and 10 literary references complete this chapter.

The Sweeteners chapter covers in detail the nutritive and synthetic sweeteners, their properties, usability in foods. Questions of health, safety and regulations are given special emphasis. 3 tables and 66 literary references are contained in this chapter.

In the Flavourings chapter in order to meet the needs of the processed food industry, the following three categories of flavours are available: full or complete flavours, flavour enhancers or modifiers, flavour extenders. The suitability of the various types of flavours (by physical form) for different end use applications; for each application, a brief description of the function of the flavours are described in tables. The chapter is completed with 2 tables, 1 figure and 7 literary references.

Colours chapter covers firts of all the properties of the various synthetic and natural colours in detail. The major colours permitted in various countries are discussed. 6 tables and 13 literary references supplement the chapter.

The aim of the Preservatives chapter is to provide the food scientists with practical information about antimicrobial preservatives for use in food products. The properties of commonly permitted antimicrobial preservatives are summarized in 2 tables and 10 literary references published just recently and are recommended for further information.

The topic of the chapter of Enzymes is to give a brief introduction to enzymology, concentrates on the parameters which affect the choice of enzymes for food application, legislation and handling. The most important enzymes are contained in 7 tables. The number of literary references is 64.

Chapter on Nutritive additives deals with the fat-soluble vitamins, water-soluble vitamins, their properties and with vitamins in food processing. Special part discusses the minerals, vitamins and mineral premixes and their government regulations. The chapter is completed with 8 tables and 7 literary references.

Chapter of Emulsifiers contains both natural and synthetic food emulsifiers. Emulsifiers made by chemical prcesses are more commonly used. 13 tables and 23 literary references supplement this chapter.

Chapter on Bulking agents covers the behaviour of bulking agents and concentrates on traditional bulking agents, bulking agents as fat substitutes and extenders and their utilization.

The pH control agents chapter gives an overview of the most frequently used acidulants in the food industry. The properties of some common food acidulants; some natural acids of fruits and vegetables are summarized in tables with special emphasis to the effect of ascorbic acid on various foods. 13 tables and 15 literary references complete this chapter.

Hydrocolloids chapter surveys the most important food hydrocolloids, their properties and fields of application. 6 tables and 7 literary references are given.

Antifoams and release agents chapter describes the products used and their physico-chemical properties. Valuable information is given by their origin and fields of application. 8 tables and 5 literary references complete the chapter.

Chapter of Flour improvers and raising agents provides very important information on all these ingredients which. have an influnece on: the formation of the gluten network; the production of CO_2 and the retention of CO_2. The main additives, their properties and their main fields of applications are contained in 12 tables. Twenty literary references complete the chapter.

Chapter of Gases deals with the question of atmosphere necessary for the shelf-life of foods. If covers freezing and chilling modified almosphere packaging. Most important information is summarized in 6 tables.

Chapter on Chelating agents deals with compounds which chelate the free metal ions in foods, and form chelates thus playing an important role in the stability and shelf-life. 5 tables and 7 literary references complete the chapter.

This handbook is useful and recommended in both research and practical technological questions.

É. SZÁNTÓ-NÉMETH

Handbook of sweeteners

S. MARIE and J. R. PIGGOTT (Eds.)

Blackie and Son Ltd., Glasgow and London, 1991, 302 pages

This handbook combines a study of sweetness with coverage of all aspects of sweeteners. For the purposes of this volume, sweetener is defined as any substance whose primary effect is to sweeten a food or beverage. All the important classes of sweeteners are included — nutritive and non-nutritive, sucrose- and non-sucrose-based, natural and synthetic, and including materials wich do not yet have regulatory approval — so that clear comparisons can be made between them and their technological advantages and disadvantages. The book is divided into eleven chapters, each chapter may be considered an individual study The first chapter — Sweetness and Food Selection — includes all the information helping to choose the sweetener or its combinations. Besides studying the sweetener — food relation, experiments among newborn infants and older infants are also reviewed. The chapter gives an overview on the newest research results through about 110 references.

In the second chapter the author deals with producing of the saccharose and its characteristics and applicability. The third chapter discusses the non-sucrose carbohydrates (honey, maple syrup and sugar, molasses and cane syrups, disaccharides other than sucrose, oligosaccharides, starch-based sweeteners, high fructose corn syrups) in a wide range. In the chapter "Sugar alcohols" useful information materials are given — besides the general review — on "digestion-absorption", "clinical tolerance" and on "energy value" as well.

The fifth chapter "Intense sweeteners" (e.g. saccharin) discusses those sweeteners which are currently available or are going to be soon available and briefly surveys some products which may appear in our food supply at some time in the future.

The next chapter (6th) covers the natural high potency sweeteners their synthetic modifications and high potency sweeteners constituted of natural sub-units (protein and non-protein sweeteners, furthermore terpenoid and polyketide sweeteners). This chapter is enhanced with 154 literature references. Special chapter is devoted in the book to sweetener markets and marketing and product development. The trends in sweetener consumption and the reason of them as they relate to consumer product marketing are reviewed in this part of the book. The relations between the sweeteners and dental health is analysed by 111 references.

The chapter "Sweeteners and metabolic disorders" describes the metabolic fate of this variety of sweet-tasting dietary components and their influence on health, dicusses the problems and diseases as diabetes mellitus and phenylketonurea. The part is completed with 117 references.

In the chapter 10 the experiments carried out for discovering relations between sweeteners and body weight are reviewed (sweeteners and body weight, mechanism of action of sweeteners on intake, theoretical implications for body weight control). The book gives a detailed information on the present regulation and on its statutory aspects and future trends.

The Handbook of Sweeteners is written for food technologists, chemists and biochemists working on food and beverage product development. Students of food science and technology and those involved with nutritional aspects of sweeteners, will find the book a valuable source of reference.

É. SZÁNTÓ-NÉMETT

Proceedings of the Third International Conference on Leaf Protein Research

Chiriotti Editori Spa, 10064 Pinerolo, Italy, 1991, 552 pages

The Leafpro-89 conference held in October, 1989 in Italy discussed the results and timely questions of leaf protein research in 3 cities, 3 sections and in 12 sessions. The first, Pisa section dealt with "Wet green crop fractionation for feed". The first and second sessions presented the world-wide leaf protein utilization and production, the third and fourth sessions the suitable crop production. Poster presentations completed the lectures, these are also included in the book. The Perugia section concerned "Leaf protein for human use" and had four sessions. The fifth session discussed Leaf protein strategy, the sixth Leaf protein technology and nutrition, the seventh the technological and nutritional applications of various leaf proteins, while the eighth, the Pharmaceutical utilization and acceptability aspects.

The motto of the Viterbo section was "Other aspects of green vegetation research". The ninth session discussed the commercial aspects of green biomasses; the tenth the Economic aspects of wet green crop fractionation, while the eleventh and twelfth the Unconventional uses of green vegetation. Similarly to the former two sections this was also supplemented by poster presentations.

The abstracts of papers of contributors who were not able to come to the Congress and present their papers are included in the Proceedings. This enables nearly the whole range discussion of the more and more important subjects.

The book gives a good survey not only for specialists of leaf protein research, processing and utilization but also for those who deal with the processing and utilization of materials of plant origin. (The price of the Proceedings is 70 US $).

I. VARSÁNYI

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Akadémiai Kiadó és Nyomda Vállalat, Budapest

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